

# Annual NIEHS/EPA Superfund



Basic Research & Training Program Meeting

# EPA/Tri University Superfund Research Program: Partnership for a Clean and Healthy Environment

October 26-27, 1998 Clark Kerr Center Berkeley, California

Hosted By

Berkeley

CDAVIS

THE UNIVERSITY OF ARIZONA,

Sponsored By

U.S. Environmental Protection Agency

National Institute of Environmental Health Sciences

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# Annual NIEHS/EPA Superfund Basic Research & **Training Program Meeting**

# EPA/Tri University Superfund Research Program: Partnership for a Clean and Healthy Environment

# Program Agenda

Monday,	October	26 <sup>th</sup>
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8:00 - 9:00 am

Registration, Check-In, Poster-Set Up, Continental Breakfast

9:00 am

Welcoming Remarks (Theater)

♦ Samuel Wilson, Deputy Director, National Institute of Environmental Health Sciences

Overview {Theater}

9:30 am

EPA Highlights, Successes and Current Problems

10:00 am

- ♦ Michael Feeley, Deputy Director of the Superfund Program University Highlights, Successes, and the Nature of Integrated Research
- ♦ Martyn T. Smith, UC Berkeley Superfund Program Director
- ♦ A. Jay Gandolfi, University of Arizona, Tucson Superfund Program Assistant Director
- ♦ Robert H. Rice, UC Davis Superfund Investigator

10:30 am

Break/Refreshments

10:30 am - Noon Poster Session (Rooms 102, 104, Theater)

Noon - 1:00 PM

Lunch (Great Hall)

1:00 - 2:00 pm

Plenary Lecture {Theater}

Risk Assessment at U.S. EPA: New Developments and Research Needs

♦ J.V. Cogliano, U.S. EPA, Washington DC

2:00 - 3:30 pm

Breakout Sessions {concurrent}

## Mines {Room 203}

Co-Facilitators

- ♦ Ned Black, U.S. EPA Region 9
- ♦ Martha Conklin, University of Arizona, Tucson
- ♦ James Hunt, University of California, Berkeley
- ♦ John Warwick, University of Nevada, Reno

## Persistent Organic Pollutants (Room 204)

Co-Facilitators

- ♦ Michael S. Denison, University of California, Davis
- ♦ Gerald Hiatt, U.S. EPA Region 9

# Perchlorates {Executive Dining Room}

Co-Facilitators

- Steven Book, CA Dept of Health Services, Office of Drinking Water
- ♦ Kevin Mayer, U.S. EPA Region 9
- ♦ Martyn Smith, University of California, Berkeley

# Remediation Technologies {Theater}

Co-Facilitators

- ♦ Mark Brusseau, University of Arizona, Tucson
- ♦ Michael Gill, U.S. EPA Region 9

3:30 pm Break/Refreshments

3:45 pm Presentation and Lecture {Theater}

The Annual Karen Wetterhahn Memorial Award

♦ Sheila Healy, Graduate Student, University of Arizona, Tucson

4:15 pm Plenary Lecture {Theater}

Airborne Technologies for the Detection of Buried Toxic Waste

♦ Roger Vickers, Director, Environmental Mapping Services

5:00 - 7:00 pm Reception (Garden Room)

# Tuesday, October 27th

8:15 - 9:00 am Continental Breakfast

9:00 - 9:30 am Opening Remarks {Theater}

- ♦ Sally Seymour, U.S. EPA Region 9
- ◆ Larry Reed, U.S. EPA Deputy Director, Office of Emergency and Remedial Response

Presentations (Theater)

9:30 am Risk Assessment at Superfund Sites: Tales from the Trenches -

Dialogue for 2000

♦ Sophia Serda, U.S. EPA Region 9

9:50 am Electrolytic Reduction: New Technologies for the Destruction of

Chlorinated Solvents

♦ Eric Betterton, University of Arizona, Tucson

10:10 am Biomarkers of Environmental Hazards to Reproductive Health and

Pregnancy

♦ Bill Lasley, University of California, Davis

10:30 am	Break/Refreshments
10:45 am	NIEHS/EPA Center for Children's Environmental Health  ◆ Asa Bradman, University of California, Berkeley
11:05 am	Molecular Analysis of Cardio-teratogenicity of Trichloroethylene (TCE)  ◆ Raymond B. Runyan, University of Arizona, Tucson
11:25 am	Biodegradation of MTBE  ◆ Daniel P.Y. Chang & Kate Scow, University of California, Davis
11:45 am	Natural Attenuation: Evaluating Needs for Superfund Sites  ◆ Mark Filippini, U.S. EPA Region 9
12:05 pm	Preventing Future Superfund Sites  ◆ Catherine P. Koshland, University of California, Berkeley
12:30 - 2:00 pm	Lunch {Great Hall} Career Fair {Executive Dining Room}  ◆ Local and regional agencies as well as industry representatives
2:00 - 3:45 pm	Breakout Sessions {concurrent}  Outreach - Risk Communication {Room 203}  Co-Facilitators  David Carpenter, State University of New York at Albany Alvin Chun, U.S. Public Health Service/EPA  Arnold Den, U.S. EPA Region 9  Technology Transfer {Room 204}  Co-Facilitators Beth Anderson, NIEHS  Michael Gill, U.S. EPA Region 9  Michael Hooper, Texas Tech University  Biomarkers/Genomics {Theater}
	<ul> <li>Co-Facilitators</li> <li>Alan Buckpitt, University of California, Davis</li> <li>Allan Smith, University of California, Berkeley</li> <li>Stanford Smucker, U.S. EPA Region 9</li> </ul>

3:45 - 4:00 pm Closing Remarks {Theater}

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# EPA/Tri-University Superfund Research Programs: Partnership for a Clean and Healthy Environment October 26-27, 1998

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# Superfund Basic Research Program Annual NIEHS/EPA Superfund Research Meeting

# EPA/Tri-University Superfund Research Programs: Partnership for a Clean and Healthy Environment

October 26-27, 1998

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# Annual NIEHS/EPA Superfund Basic Research & Training Program EPA/Tri University Superfund Research Program: Partnership for a Clean and Healthy Environment

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Tuesday, October 27, 1998 12:30 -2:00 pm Clark Kerr Center Berkeley, California

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SPEAKER PRESENTATIONS

# Sheila Healy, Graduate Student, University of Arizona, Tucson Recipient of the First Annual Karen Wetterhahn Memorial Award

ENZYMATIC METHYLATION OF ARSENIC IN THE GUINEA PIG AND MOUSE.

Arsenite and monomethylarsonate (MMA) methyltransferase activities >were assessed in guinea pigs by incubating liver cytosol with sodium arsenite or MMA and S-[methyl-3H]-adenosyl-L-methionine and measuring the formation of [methyl-3H]-MMA or [methyl-3H]-dimethylarsinate (DMA), respectively. Guinea pig cytosol did not catalyze the formation of these methylated metabolites. Moreover, when guinea pigs were given a single intraperitoneal dose of [73As]-arsenate and urine collected 0-12 and 12-24 hours after injection, 98 and 93% of the excreted dose was inorganic arsenic, respectively. These results indicate that, unlike any other laboratory animal model, the guinea pig does not detoxify arsenic via methylation.

By quantitating [methyl-3H]-MMA, in vitro enzyme activity was also determined in B6C3F1 mice. The following mean arsenite methyltransferase specific activities (U/mg SEM) were measured: liver, 0.40 0.06; testis, 1.45 0.08; kidney, 0.70 0.06; and lung, 0.22 0.01. Heretofore, enzymatic methylation of arsenic has been regarded primarily as a hepatic function. However, these data show that the arsenite methyltransferase specific activity of the testis was 3.6 times greater than that of the liver (p < 0.01) and the specific activity of the kidney was 1.8 times greater than that of the liver (p < 0.05).

# INNOVATIVE TECHNOLOGIES FOR THE DEHALOGENATION OF CHLORINATED ORGANIC SOLVENTS

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Heavily halogenated organics such as carbon tetrachloride and trichloroethylene represent a class of highly oxidized molecules that are thermodynamically amenable to reduction to the parent aliphatic with elimination of halide ion. They can also be induced to undergo substitution reactions resulting in conversion to CO2. Here we review recent progress in electrochemical and photochemical methods for driving dehalogenation reactions in aqueous solution and also in the gas phase. Mechanistic and kinetic models were developed for electrolysis of both aqueous- and gas-phase halogenated aliphatic compounds. Reaction rates were dependent on electrode material, electric potential and certain reactor-dependent characteristics. In the absence of mass-transport limitations, first-order rate constants were inversely related to the carbon-hydrogen bond energy of the target compound. In most experiments, reaction stoichiometry was (essentially) completely accounted for. It proved possible to select electrode materials and cathode voltages that would permit reductive dehalogenation to proceed without excessive hydrogen gas generation.

For the first time, we describe the results of gas-phase electrochemical reduction in a 2-compartment fuel cell. Rates are the fastest we have observed in any laboratory system. Also, rapid photochemical reductive dehalogenation of aqueous carbon tetrachloride is possible in the presence of a carbonyl chromophore (e.g., acetone) and a suitable H-donor (e.g. ethanol). The kinetics and mechanism of this rapid and apparently novel system will also be discussed.

Continued work is aimed at the development of new, practical means to decontaminate polluted soil and ground water.

#### CHILDREN'S CENTER FOR ENVIRONMENTAL HEALTH RESEARCH

Ada Bradman, University of California, School of Public Health, Berekley CA

The goal of this Center is to establish a forum for the multidisciplinary approach to understand and reduce the environmental health risks of children. This Center is a joint partnership between the University, the local and State health departments, community clinics, and our community organization, South County Outreach Effort (SCORE). We have brought together a diverse team of specialists representing many disciplines to address the special environmental issues of children.

For this study, we have focused on the environmental risks of a largely underserved population of latino farmworkers in Salinas Valley in Monterey County, California. California, with the largest agricultural output of the 50 states, uses 25% of the national total (over 210 million pounds) of pesticides annually, with over 725,000 pounds of organophosphate (OP) pesticides used in the Salinas Valley alone. Furthermore, approximately 1 million California residents are farmworkers, with Monterey County leading the numbers.

Recently, several studies have demonstrated pesticide contamination in children's homes from agricultural areas. It is estimated that 20-60% of children aged1 to 5 years may be exposed to unsafe levels of OP pesticides. Nevertheless, to date, few studies have assessed the extent of children's pesticide exposure, and no studies have examined whether there are adverse health effects of low-level chronic exposure. Farmworker children are exposed by the usual pathways, i.e., by ingestion of contaminated foods and from home pest control, as well as through pesticide drift from nearby fields and from the take-home exposures of their parents. Studying this high-risk population, we can ascertain whether chronic, low-level exposures may be potentially hazardous.

We will interview and bank biologic and environmental samples from a cohort of 550 pregnant women and their children, who will be followed for 3 years. Our specific aims are: 1) to estimate levels of *in utero* and postnatal exposures of the child by measuring biologic and ambient samples; 2) to determine whether exposure to pesticides is associated with adverse effects on neurodevelopment and growth, and increased respiratory disease including asthma; and 3) to initiate and evaluate the impact of a 'Healthy Homes' intervention on the reduction of pesticide exposure to children.

Our ultimate goal is to translate research findings into a reduction of pesticide exposure to children, and to reduce the incidence of environmentally related childhood disease.

#### **BIODEGRADATION OF MTBE**

J.B. Eweis<sup>1</sup>, J.R. Hanson<sup>2</sup>, <u>K.M. Scow</u><sup>2</sup>, <u>D.P.Y. Chang</u><sup>1</sup> and E.D. Schroeder<sup>1</sup>, <sup>1</sup>Department of Civil & Environmental Engineering, <sup>2</sup>Department of Land Air & Water Resources, University of California, Davis

The gasoline oxygenate methyl tert-butyl ether (MTBE) is now one of the most widely produced organic chemicals in the world. Its widespread appearance in surface and groundwater is well-documented, but little evidence for its biodegradability in natural systems has been validated. Efforts over the past two years at the University of California, Davis have resulted in the culture of a naturally occurring microbial consortium that rapidly degrades MTBE aerobically. The consortium has been successfully transferred to engineered gas-phase air treatment units, aqueous attached and suspended growth bioreactors, as well as to saturated sand environments. The MTBE-degrading bacterial isolates and associated consortia have also been characterized. Efforts are underway to begin field-testing of both ex situ and in situ systems.

Two bacterial strains, PM1 and YM1, which can use MTBE as their only source of carbon and energy, were isolated from the MTBE-degrading microbial consortium from a compost biofilter. MTBE degradation by PM1 is rapid, with complete disappearance of 25 ug MTBE per ml within 4 days. YM1 degrades MTBE more slowly (50% degraded in 4 days). Examination of whole cell lipid profiles for PM1 and YM1 suggest they are not identical strains. Thermal gradient gel electrophoresis (TGGE) analysis of 16S rRNA gene fragments from the two strains, however, indicates no difference between them. The 16S rDNA sequence analysis of strains PM1 and YM1 reveal that they are both most closely related to the genus Sphingomonas. Fingerprinting methods that analyze cell components (e.g. phospholipids, DNA) extracted directly from mixed cultures and environmental samples can provide insight into the composition and stability of microbial communities that degrade MTBE. Analysis of DNA by TGGE indicated that the two bacterial isolates described above are members, but do not appear to be the most dominant members of the MTBE-grown microbial consortium. An additional unidentified band was particularly dominant when the consortium was cultured on MTBE. This 16S rDNA band has been sequenced, and its sequence is most closely related to the 16S rDNA of the bacterial genus Rubrivivax.

# RISK ASSESSMENT AT THE U.S. EPA: NEW DEVELOPMENTS AND RESEARCH NEEDS

Jim Cogliano, United States Environmental Protection Agency

Risk assessment at EPA is changing dramatically, responding to several new developments. EPA's new cancer guidelines will encourage risk assessments to discuss mode of action. New emphasis on human heterogeneity will promote consideration of groups that may be at higher risk, especially children. Congress and community groups are encouraging EPA to expand risk assessments to consider multiple pollutants from multiple sources and multiple pathways. These changes signal a new era in risk assessment, where procedures that tend to overestimate risk would be replaced by a presumption to use more of the available information and, it is hoped, lead to more likely estimates of risk.

# NATURAL ATTENUATION: EVALUATION NEEDS FOR SUPERFUND SITES

Mark Filippini, Hydrogeologist, U.S. EPA Region 9, 75 Hawthorne St., Mail Code SFD-8B, San Francisco, CA 94105-3901.

As Monitored Natural Attenuation (MNA) becomes a more recognized potential remedy for Superfund sites nationwide, the technical evaluation requirements for making an adequate demonstration will need to be considered. Superfund technical managers must evaluate whether MNA is actually occurring at their sites, and what additional technical data must be collected in order to make an adequate technical demonstration. Once data is obtained, evaluation of the data through computer modeling and demonstration of likely biochemical processes must be performed. This presentation will focus on some of the factors to consider while planning and implementing field investigations to assure that adequate data is obtained and the data is properly evaluated.

#### PREVENTING FUTURE SUPERFUND SITES

Catherine P. Koshland, Wood-Calvert Professor in Engineering, Environmental Health Sciences, School of Public Health, and the Energy and Resources Group, University of California, Berkeley

One of the lessons learned from the Superfund program is the value of prevention: anticipating problems in waste generation and disposal can reduce the incidence of hazardous events or development of sites that pose chronic exposure problems. Yet strategies to identify design flaws in products or processes, to anticipate the generation of wastes in manufacturing or energy production, to manage materials flows with respect to environmental and human health hazards are still in their infancy. Incorporating environmental metrics and standards into product and process design adds a dimension of complexity that increases initial costs; yet those very steps may reduce the need for even more costly "end - of- pipe controls" or reduce the costs of hazardous materials management and disposal or reduce the costs of Superfund type cleanups.

Much of the research devoted to Superfund has been targeted to remediation and cleanup of existing Superfund sites, and on identifying markers of exposure to the chemicals present in those sites. Much less attention has been devoted to the technical, engineering, management and economic decisions that lead to the generation of hazardous materials, their use and disposal. As a material or products is developed, the emphasis is its performance for its intended function, and much less on the consequences of the manufacturing process in terms of waste generation, intermediate materials or byproducts, and the potential for exposure to these materials. Several approaches including Life Cycle Assessment, scoring systems, and environmental metrics for design applications are being proposed. How, when and on what scale to apply these different methods of evaluation is an on-going question worthy of study.

# A NEW BIOMARKER FOR EVALUATING EARLY FETAL LOSS IN POPULATION BASED STUDIES

B.L. Lasley, S.H.Ho, E.Enan, Y.Guo, D.R. Stewart, G.C. Douglas, and J.W. Overstreet, Institute of Toxicology and Environmental Health, University of California

As many as 50% of all human conceptions end in abortion and as much as 30% of all of these abortions occur without being recognized by either the woman or her physician. Most of these unrecognized early fetal losses (EFLs) are probably the result of chromosomal abnormalities and represent the expected natural attrition in a process that is prone to error. Some of these losses, however, may be the result of adverse effects of exposures to environmental hazards, but current methods which detect EFLs using endocrine biomarkers cannot distinguish between those that are predestined to fail spontaneously and those that would have survived had an environmental hazard not intervened. To date no EFL that resulted from a toxic exposure has been characterized and, while we believe this aspect of reproduction is highly vulnerable to toxic exposure, it is not clear if EFLs are, in fact, induced by environmental hazards.

Recent work in our laboratories has demonstrated that there are differences in the biological activity of THE human chorionic gonadotropins (hCG) that are produced initially and then later during the first two weeks of pregnancy. In addition, the pattern of change in hCG bioactivity during the first two weeks following conception is predictive of the pregnancy outcome and provides for the first time a method of assessing the health of a conception during the time that many pregnancies fail spontaneously. On the basis of these findings, we hypothesize that the assessment of peri-implantation hCG bioactivity profiles will identify pregnancies that are normal at the time of implantation and therefore are at risk when exposed to environmental hazards. Such categorization may enable us to distinguish natural, spontaneous abortions from environmentally-induced pregnancy loss.

Using TCDD as a model compound to induce pregnancy loss in the laboratory macaque, we demonstrated that following TCDD exposure and preceding expulsion of the fetus there was a change in the bioactivity of monkey chorionic gonadotropin (mCG). Similarly, when treated in vitro with TCDD, human trophoblast cells secreted hCG with altered bioactivity. Taken together, these data demonstrate that measurement of the bioactivity of chorionic gonadotropin during very early pregnancy provides an assessment of the health of that pregnancy and is predictive of pregnancy outcome. Toxicants which cause EFL may also cause a shift in the bioactivity of hCG at the time of exposure and the change in hCG bioactivity may be an indicator of the adverse effects of an environmental hazard in an otherwise normal pregnancy.

# MOLECULAR ANALYSIS OF CARDIO-TERATOGENICITY OF TRICHLOROETHYLENE

J. Michael Collier, Cathleen Cover, Angelique S. Boyer, Wendy T. Finch, Paula D. Johnson, Ornella Selmin and <u>Raymond B. Runyan</u>, <u>Department of Cell Biology & Anatomy, University of Arizona College of Medicine</u>, <u>Tucson AZ</u>

Previous studies in human and animal populations suggested that the halogenated compound, trichloroethylene (TCE), is a heart-specific teratogen. To study the apparent sensitivity of the heart to TCE, two research directions were explored. In one approach, a tissue culture model of cardiac valve formation was exposed to various doses of TCE. Epithelial-mesenchymal cell transformation (the process that starts formation of heart valves) in embryonic chick heart explants was inhibited by TCE (50-250 ppm). Valvular progenitors were reduced by up to 50% because endothelial cells failed transform. Analysis of marker expression showed a regulatory transcription factor, mox-1, critical to cell transformation, to be specifically inhibited by TCE. Concurrent studies were performed pregnant rats exposed to varying doses of TCE, DCE or TCAA in drinking water. Day 11 embryos (during heart valve development) were collected and total heart RNA was pooled. cDNA libraries were prepared for each group and used in a PCR-based subtraction hybridization to identify up- and down-regulated gene expression. Candidate clones were rescreened to confirm differences in expression and sequenced. To date, more than 150 clones have been sequenced. Many clones were perturbed by each of the halogenated reagents in similar fashion. Altered genes included stress proteins, RNA processing molecules and housekeeping genes. Several cytoskeletal and cell surface molecules were also perturbed. A few identified molecules were used for in situ hybridization to explore localized expression in embryos. One molecule, p137, was specifically expressed in the embryonic heart muscle and was up-regulated by exposure to 110 ppm TCE. These studies suggest that specific molecular and cellular targets can be identified for TCE.

# RISK ASSESSMENT AT SUPERFUND SITES: TALES FROM THE TRENCHES-DIALOGUE FOR 2000

Sophia Serda, U.S. EPA Region 9, 75 Hawthorne St, Mail Code SFD-8B, San Francisco, Ca 94105-3901.

Superfund baseline risk assessments are conducted to evaluate potential human health and environmental risks posed by an uncontrolled hazardous waste site. The results are critical in determining whether or not response actions are justified and are used as a basis for taking appropriate measures to protect human health and the environment. The assessment process is also used to identify potential risks associated with a particular remedy and to evaluate risks remaining at a site when the cleanup is done. Specifically, the presentation will provide suggestions for how technical staff and community members can work together during all stages of a Superfund cleanup; identify where scientific input can augment and improve the estimated exposures and risks within the framework of the human heath risk assessment methodology and suggests ideas for the future of risk assessment at Superfund sites.

#### AIRBORNE TECHNOLOGIES FOR DETECTION OF BURIED TOXIC WASTE

Roger S Vickers, Director, Environmental Mapping Services

Airborne screening of areas believed to be contaminated with buried or surface toxic waste can offer significant cost and time savings in the right circumstances. The advantages of airborne survey are obvious, - large stand-off distance, access to the areas without permits, large rate of aerial survey. Unfortunately, the disadvantages are equally striking. The technologies may not always work, urban build-up frequently make the methods impossible to apply, and the surveys can be limited by weather, FAA restrictions, and cost. Nevertheless, any method that reduces the amount of work that has to be performed on the ground is worthy of attention.

The emphasis of this talk will be on the use of airborne ground penetrating radar in the search for buried toxic waste. The differences in performance when using GPR in the profiling mode as opposed to the imaging mode will be explained. The use of other sensors, including hyperspectral and thermal infrared imagers will also be mentioned.

Dr Vickers was educated in the UK and obtained his PhD in Physics in 1965. Since coming to the USA, he has worked for about ten years in optical remote sensing techniques, and the last twenty years in ground penetrating radar. He has been on the EE faculty at Colorado State University, the research staff at Stanford University, and most recently at SRI International. He is currently working with a start-up company in the business of remote sensing of buried targets, with particular emphasis on the problem of humanitarian landmine clearance.

#### TECHNOLOGY TRANSFER BREAKOUT

Tuesday, October 27, 1998, Beth Anderson, National Institutes of Environmental Health Sciences; Michael Gill, U.S. EPA; Michael Hooper, Texas Tech University, Co-Facilitators

The focus of this breakout session will be to advance the exchange of information between the NIEHS/EPA Superfund Basic Research Program grantees and EPA Region IX staff. Specific emphasis will be given to developing creative strategies for transferring information in a timely and effective manner, identifying the types of information that are appropriate and desirable for transfer and identifying points of contact within each group to facilitate the transfer of information. An example of a successful transfer of research to application at an NPL site will be presented to demonstrate what one researcher has learned from this process. The session will include ample time for the breakout participants discuss opportunities for information transfer and develop novel approaches that can be implemented by NIEHS, SBRP grantees or EPA.

### POSTER PRESENTATIONS

### FORECASTING GROUNDWATER BIOREMEDIATION IN COMPLEX GEOLOGIC SYSTEMS

Christopher T. Green and Graham E. Fogg, Department of Land Air and Water Resources, University of California, Davis

Natural and enhanced bioremediation have gained popularity due to their relatively low cost and ease of application. However, biological and physical factors often limit success, and field practitioners face the quandary of evaluating remediation alternatives based on tenuous predictions of efficacy. Biodegradation can be numerically modeled, but paucity of data usually leads to spatial averaging of parameters, which causes erroneous predictions. The magnitude and character of these errors is unresolved. Hence, there is an immediate need to assess the effects of heterogeneity and spatial averaging on the prediction of field scale biodegradation of contaminants.

Recent advances in geostatistical simulation, modeling of advection/diffusion processes, and conceptual models of microbial distribution in the subsurface were used to test: 1) whether correlation of microbial activity with geology influences the migration pattern of a pollutant; and 2) whether the use of an average decay coefficient leads to consistent overestimation of total biodegradation (as reported in previous studies). Different scenarios of decay coefficient distribution were assigned within a geostatistical simulation, and contaminant transport was modeled using random walk particle tracking. In these experiments, correlation of microbial activity with geology had dramatic effects on a plume's center of mass, total mass, and maximum concentrations. These effects varied with the type of correlation, and the distance from the source. In addition, our experiments showed that (contrary to existing studies) spatial averaging of decay coefficients can cause underestimation of biodegradation. These findings indicate a need for detailed characterization in evaluation of remediation alternatives.

#### HPLC-AMS MEASUREMENT OF ATRAZINE METABOLITES IN HUMAN URINE

Bruce A. Buchholz\*, Kurt W. Haack\*, John S. Vogel\*, S. Douglas Gilman†, Shirley J. Gee†, Bruce D. Hammock†. \*Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory & †Department of Entomology, University of California, Davis.

Human metabolites of atrazine were measured in urine after dermal exposure using HPLC to separate and identify metabolites and accelerator mass spectrometry (AMS) to quantify them. Ring labeled "C-atrazine was applied for 24 h with a dermal patch to human volunteers at low (0.167 mg, 6.45 mCi) and high (1.98 mg, 24.7 mCi) doses and urine was collected for seven days. The urine was centrifuged to remove solids and the supernatant was measured by LSC to insure that <10 DPM was injected on the HPLC column. The mobile phase gradient became less polar with increasing time and separated the parent compound and major metabolites over 31 minutes. Peaks were identified by co-elusion with known standards. Elution fractions were collected in 1 min increments and half of each fraction (0.500 mL) was prepared for analysis by AMS. Isotope ratios were measured by AMS to ±3% for all samples. Mercapturate metabolites of atrazine and dealkylated atrazine dominate the early metabolic time points, accounting for ~90% of the "C in the urine. No parent compound was detected. The mix of metabolites became more polar after removal of the dosing patch. At Day 7, an unknown polar metabolite that was present in all samples became as prevalent as any of the known ring metabolites. Knowledge of metabolite dynamics is crucial to developing useful assays for monitoring atrazine exposure in agricultural workers. Work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under contract W-7405-ENG-48, and NIEHS ES04699 at the University of California, Davis.

# STEAM TREATMENT OF PCB CONTAMINATED SOIL INNOVATIVE OUTREACH PART III

Mark Hutchinson, Daniel P.Y. Chang, and Pingkuan Di, Department of Civil and Environmental Engineering, University of California, Davis, and Kent S. Udell and Michael Itamura, Department of Mechanical Engineering, University of California, Berkeley

Steam injection technologies have been used by the petroleum industry to aid removal of crude oil. Professor Kent Udell and his students at the University of California, Berkeley have pioneered the use of steam injection for remediation of soils contaminated with volatile contaminants such as gasoline, jet fuel and diesel. The present study is the third report of a series of tests of the steam injection process for removal of polychlorinated biphenyls (PCBs) from contaminated soils at the former Mare Island Naval Shipyard (MINSY), Vallejo, CA. The removal of approximately 86% of the PCBs from a contaminated soil at MINSY was achieved. The study involved collaboration of the U.S. Navy's SSPORTS Environmental Detachment (SSPORTS/ED) and the University of California Berkeley (UCB) and Davis (UCD) campuses' Superfund program projects as part of an "outreach" effort.

Civilian engineers employed at MINSY were first taught principles of steam injection through a lecture course. The design of a bench-scale ex situ steam treatment system, adapting in situ steam injection techniques, was a product of the laboratory portion of the course. Previous lab-scale (50 g samples) experiments effectively displaced PCBs from contaminated soils, with removal efficiencies as high as 98% for an Aroclor 1260 at steam temperatures as low as 150° C. The ex situ bench-scale treatability study set the stage for the PCB (B.P. 275-385° C) by initially removing 85% of "surrogate PCBs" octadecane (B.P. 317° C) and chrysene (B.P. 448° C). The treatment process consisted of cyclically driving steam through approximately 10 kg samples of contaminated soil contained in a pressure vessel at temperatures and pressures reaching as high as approximately 150° C and 60 psig.

# 1-NITRONAPHTHALENE GLUTATHIONE CONJUGATION IN RAT AIRWAYS AND LIVER

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<sup>1</sup>Dept. of Molecular Biosciences, School of Veterinary Medicine; <sup>2</sup>Dept. of Chemistry; 
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1-Nitronaphthalene (1-NN) is a mutagenic nitroaromatic which has been detected in emissions from both heavy- and light-duty diesel engines, as well as in urban airborne 1-NN is a cytochrome P450 (P450)-bioactivated, non-ciliated bronchiolar epithelial (Clara) cell cytotoxicant. The current studies examined the metabolism of 1-NN to electrophilic metabolites which were trapped as glutathione conjugates in highly susceptible (lung) and less susceptible (liver) tissues in rat. Significant depletion of reduced glutathione was observed in all levels of tracheobronchial airways of rats treated with 200 mg/kg 1-NN, ip. This was consistent with the HPLC radioactivity profiles demonstrating five putative glutathione conjugates isolated from liver microsomal incubations with 1-NN, glutathione S-transferase and tritiated [3H] glutathione. Mass spectrometry in positive ion mode yielded a m/z of 497 (M+H), and daughter ions of m/z 479 (loss of water) and m/z 306 (glutathione), which were consistent with the formation of hydroxy-dihydro-glutathionyl derivative of 1-NN. Proton nuclear magnetic resonance (NMR) spectroscopy analysis suggested the formation of two major conjugates from liver microsomal incubations. 1-nitro-5-glutathionyl-6-hydroxy-5,6namely dihydronaphthalene and 1-nitro-5-hydroxy-6-glutathionyl-5,6-dihydronaphthalene. These data show that P450 metabolizes 1-NN in the liver to an intermediate 5,6-epoxide, which is further metabolized by glutathione S-transferase to form 1-NN glutathione conjugates. HPLC radioactivity profiles demonstrate that major conjugate(s) generated in the lung differ from those generated in the liver, thus suggesting that either the intermediate epoxide is formed at different carbon positions, or the stereoisomer of the liver conjugate is formed in the lung.

### ENHANCED DETECTION OF PLASMID pJP4 TRANSFER IN 2,4-D CONTAMINATED SOILS

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Transfer of a large catabolic plasmid, pJP4, from an introduced organism to indigenous soil recipients was examined. Genes on this plasmid encode for mercury resistance and for partial 2,4-dichlorophenoxyacetic acid (2,4-D) degradation. Plasmid pJP4 was introduced into Escherichia coli (ATCC 15224), via plate mating with Alcaligenes eutrophus JMP134. The resultant E. coli donor lacks the chromosomal genes necessary for the mineralization of 2,4-D. This allows for the selection of presumptive indigenous recipients (transconjugants) via plating on media with 2,4-D as the carbon source. In previous studies, using A. eutrophus JMP134 as the donor organism, gene transfer was only detected in Madera Canyon soil amended with 1,000 µg of 2,4-D g dry soil<sup>-1</sup>. Preliminary studies using the E. coli donor showed gene transfer in Madera Canyon, Bear Canyon, and Rose Canyon soils amended with 500 µg of 2,4-D g dry soil<sup>-1</sup> and in the first two soils with 1,000 µg of 2,4-D g dry soil<sup>-1</sup>. In an expanded study, the E. coli donor was inoculated into triplicate nonsterile microcosms containing Madera Canyon soil amended with either 500 µg or 1,000 µg of 2,4-D g dry soil<sup>-1</sup>. Populations of presumptive transconjugants were found and confirmed: 10<sup>7</sup> and 10<sup>8</sup> g dry soil<sup>-1</sup> for 500 µg and 1,000 ug amendments respectively. No mercury-resistant, 2,4-D degrading organisms with large plasmids or the tfdB gene of plasmid pJP4 were found in the 2,4-D amended but uninoculated control microcosms. In addition, slightly enhanced rates of 2,4-D degradation were observed in microcosms supporting transconjugant populations relative to controls in which no gene transfer was detected. These results suggest that donor counter-selection enhances the detection of plasmid pJP4 transfer to indigenous soil populations.

### CO-INOCULANT MICROBIAL REMEDIATION OF METAL:ORGANIC CONTAMINATED SOILS

<u>Timberley M. Roane</u>, Karen L. Josephson and Ian L. Pepper. Department of Soil, Water and Environmental Science The University of Arizona, Tucson

Organic-degrading microorganisms are common in the scientific literature, as are metalresistant microorganisms. Yet, few studies have addressed the use of organic-degrading microorganisms in conjunction with metal-resistant microorganisms in the remediation of systems co-contaminated with both organic and metallic pollutants. Current thinking suggests that degradation does not occur in co-contaminated environments because of the toxicity of the metal. Identified by BIOLOG metabolic fingerprinting as *Pseudomonas*, Bacillus and Corynebacterium spp., several cadmium-resistant (up to 200 ppm soluble cadmium) isolates from environmental soil samples were found to detoxify cadmium. This cadmium detoxification allowed for cadmium-sensitive Alcaligenes eutrophus JMP134 to mineralize 500 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) in the presence of up to 50 ppm of bioavailable cadmium. Without the addition of a cadmiumdetoxifying isolate, 2,4-D degradation did not occur within the time frame of the experiments. Both a cadmium-detoxifying population and a 2,4-D degrading microbial population were necessary to support degradation under co-contaminated conditions in pure culture, soil microcosms and intermediate field scale bioreactors. Preliminary data suggest that gene transfer was occurring in these co-contaminated systems resulting in 2,4-D degrading and/or cadmium-resistant transconjugants. In 2,4-D only contaminated soils, we have demonstrated enhanced 2,4-D degradation upon the transfer of the plasmid pJP4, encoding 2,4-D degradation, from the A. eutrophus JMP134 donor to indigenous recipients. This study demonstrates the potential use for co-inoculation of metal-resistant and organic-degrading microorganisms in the remediation of complex contaminated environments.

ARSENITE [As(III)] AND ARSENATE [As(V)] ENHANCE DNA BINDING OF THE AP-1 AND Elk-1 TRANSCRIPTION FACTORS AND c-jun AND c-fos GENE EXPRESSION IN PRECISION-CUT RENAL SLICES.

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Although arsenic is recognized as a nephrotoxicant, little is known about the molecular impact of low concentrations of various arsenic species on the kidney. Our previous studies have shown that nanomolar concentrations of arsenite [As(III)] or arsenate [As (V)] do not elicit overt cytotoxicity, but are associated with a concentration- and timedependent induction of heat shock protein 32 (Hsp 32; heme oxygenase-1). Given that Hsp32 is regulated, in part, by the activator protein-1 (AP-1) transcription factor, we hypothesized that As(III) and As(V) would activate the MAPK pathway. transcription factors lie downstream of the stress-activated c-jun N-terminal kinase and p38 kinase: AP-1, ATF2 and Elk-1. Using electrophoretic mobility shift analysis, the impact of As(III) and As(V) on the DNA binding of these factors was investigated (0-1000 nM; 2-6 hr). Both species enhanced the DNA binding of AP-1 and Elk-1, but did not effect DNA binding of ATF2. Since expression of the early-immediate genes c-jun and c-fos are driven by AP-1 and Elk-1, respectively, expression of these genes was examined by RT-PCR. After 2 hr of exposure, 100 nM As(III) or As(V) enhanced c-jun gene expression 1.32±0.07 and 1.20±0.04 fold, respectively. c-fos gene expression was enhanced 1.44+0.12 and 1.78+0.16 fold by 100 nM As(III) and As(V), respectively, at 2 hr. These results demonstrate that both As(III) and As(V) elicit a specific activation of molecular signaling pathways in renal tissue at low concentrations. Supported by NIH ES04940 and an SOT Colgate-Palmolive Fellowship.

# SELECTIVE ACTIVATION WITHIN THE MAPK PATHWAY IN RENAL TISSUE BY HG(II)

K.D. Turney, A.R. Parrish, A.J. Gandolfi. Department of Pharmacology and Toxicology, University of Arizona

The kidneys are the primary target organ for the accumulation and toxicity of inorganic mercury. Studies have shown molecular alterations occur following a nephrotoxic insult, thus alterations in transcription factor binding and gene expression may be involved in the response of the kidney to metals. However, little is known about the molecular response of the kidney to environmentally relevant concentrations of Hg(II). Previous research in our laboratory utilizing rabbit renal cortical slices demonstrated that noncytotoxic concentrations of Hg(II) (0-1000 nM), increased the DNA binding activity of the AP-1 transcription factor which correlated with a dose-dependent enhancement of cjun gene expression. These results suggest a possible interference in the c-Jun NH<sub>2</sub>terminal protein kinase (JNK) pathway. Therefore the effects of Hg(II) on other components of this pathway were investigated. No changes were observed in the DNA binding activity of ATF2 and Elk-1, transcription factors involved in both the JNK and p38 pathways of MAP signal transduction. The specificity of Hg(II) for MAP signal transduction was also demonstrated by no changes of c-myc gene expression after exposure to Hg(II). The selectivity of alterations in molecular signaling suggests an acute response in signal transduction, specifically activation of the JNK pathway, in renal tissue following exposure to nanomolar concentrations of Hg(II). (NIH ES04940, NIEHS Training Grant T32 ES07091)

# HOW CLEAN! RADIOACTIVE CONTAMINATION CLEANUP FROM A CERCLA/SUPERFUND PERSPECTIVE.

Stephen M. Dean, U.S. EPA Region 9, San Francisco, CA 94105-3901

US EPA's Office of Emergency and Remedial Response (OERR) has declared that radionuclides are not "privileged pollutants". Under CERCLA all radionuclides are Group A carcinogens and must be addressed using cancer risk assessment and Preliminary Remediation Goal (PRG) methodologies similar to those used for toxic chemicals. This presentation provides information on how to evaluate radionuclides of concern at radiation contaminated sites using the Risk Assessment Guidance for Superfund (RAGS). Practical methods for risk assessment and for calculating PRGs will be discussion topics.

#### ANODIC OXIDATION OF TRICHLOROETHYLENE AT A CERAMIC ANODE.

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Halogenated organic compounds can be destroyed via oxidation reactions in systems that are capable of yielding oxidizing radicals such as hydroxyl (OH $^*$ ). Such systems include electrolytic reactions in which OH $^*$  is generated in the anodic compartment. In this work, the electrolytic reactor consisted of two compartments that were separated by a cation-exchange membrane. The working electrode (anode) was constructed from Ebonex<sup>(R)</sup>, a conducting ceramic comprised primarily of  $Ti_4O_7$ .

In these experiments, trichloroethylene (TCE) was oxidized on Ebonex anodes. Carbon-containing products were primarily  $CO_2$  and traces of CO. There were no other major carbon-containing products. At neutral and alkaline pHs, the only chlorine-containing products were  $Cl^-$  and  $ClO_3^-$ . The rate of TCE disappearance was established as a function of the fixed anodic potential, bulk solution pH and TCE concentration. Under our experimental conditions ( $E_{anode}=2.5$  to 4.3 V vs. SSCE), the disappearance of TCE was first-order in TCE concentration. Transformation rate was independent of pH in the range 1.6 < pH < 11 and TCE initial concentration in the range  $1 \mu M \le C_{TCE} \le 1 \mu M$ . TCE oxidation occurred only on the anodic surface and was limited by mass transport at high potentials ( $E_a > 4.0 \text{ V}$ ). The maximum (transport - limited), surface-area-normalized rate constant was about 0.13 cm/min. The current efficiency for TCE oxidation was inversely related to the anode potential. Hydroxyl radicals were produced at the anode surface and detected using the spin-trap technique. The effect of electrolyte ionic strength on TCE anodic oxidation was experimentally investigated.

A mechanism for the anodic reaction was proposed, and model kinetics was successfully correlated with experimental results.

# PHOTOLYTIC REDUCTION OF CHLORINATED ALIPHATICS IN MIXED SOLVENTS

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Light-driven reductive transformation of carbon tetrachloride (CCl<sub>4</sub>), chloroform (CHCl<sub>3</sub>), perchloroethene (PCE), trichloroethene (TCE) and other short-chain haloaliphatic compounds was observed in homogeneous mixtures of acetone, 2-propanol and water. The transformation of CCl<sub>4</sub> to CHCl<sub>3</sub> was light-limited and zero-order in CCl<sub>4</sub>. Reduction of CCl<sub>4</sub> in mixtures of benzophenone or acetophenone in ethanol is currently under investigation. Reduction of the halogenated compounds did not take place in the absence of either the alcohol or ketone. The reactions were driven by near ultraviolet light (200 ≤λ≤ 450nm). In direct sunlight, millimolar levels of CCl<sub>4</sub> in borosilicate glass containers were reduced to near-zero concentrations in about 5 minutes. The products of TCE transformation reactions were not positively identified, and did not include dichloroethene isomers or vinyl chloride. The presence of oxygen results in a lag phase preceding the reduction of CCl<sub>4</sub>, which is then reduced at a much faster rate than in systems that are purged with inert gas. The lag phase has been attributed to the reduction of oxygen to hydrogen peroxide. A mechanism was suggested for the reductive dechlorination of CCl<sub>4</sub> in the acetone/2-propanol system. Light energy is first absorbed by the acetone chromophore. Hydrogen atom abstraction by acetone in its excited state from 2-propanol results in two identical ketyl radicals ((CH<sub>3</sub>)<sub>2</sub>COH·). Subsequent oneelectron transfer from the radicals to CCl<sub>4</sub> yields trichloromethyl radical, which is then transformed to CHCl<sub>3</sub> via hydrogen abstraction. The proposed chain reaction accounts for quantum efficiencies considerably greater than one. We know of no other reports of light-driven reductions of halogenated aliphatic compounds in homogeneous solutions consisting of ketone, alcohol and water.

#### DUST RESUSPENSION AS A CONTAMINANT EXPOSURE PATHWAY

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Numerous environmental contaminants sorb to dust particles or exist as particles, including metals, hydrophobic organic compounds, asbestos, pollens, and microbial pathogens. Wind resuspension of dust and other particulate matter provides a transport route for contaminants and therefore a risk to human health. In addition, resuspended dust particles are believed to play a role in global climate change and chemical atmospheric reactions. The conditions under which contaminated sites are vulnerable to wind resuspension are not generally known, as the basic physics of the problem are poorly understood. Field data show tremendous variability.

Conventional dust flux models assume that dust resuspension occurs only for high wind events and then only for a brief time, on the order of minutes, with a short-term transient dust flux occurring only when the bed is first exposed to the high wind. Recent wind tunnel experiments demonstrate that surfaces yield continuous steady dust fluxes under steady wind conditions well beyond the initial high transient flux, even when no erosion is visible and the velocity is below the predicted threshold velocity for movement. This average steady-state dust flux increases with average wind speed. Ongoing work is investigating the influence of air relative humidity on these processes.

Current models to predict the resuspension flux of contaminants in the environment at best indicate trends. Risk analyses require better predictive modeling, necessitating a deeper understanding of the underlying phenomena. More data are needed both to elucidate the basic processes and to identify sites from which contaminant and dust resuspension pose threats to human health and the environment.

# TRACING CHEMICALS IN ANIMALS OR ENVIRONMENTS AT ATTOMOLE SENSITIVITY

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Accelerator Mass Spectrometry (AMS) quantifies rare isotopes, usually radioactive, at parts per billion to parts per quadrillion, measuring attomole (10-18 mole) quantities of isotope labeled compounds in mg-sized samples. We have traced radiocarbon and tritium labeled toxins, nutrients, and therapeutics in human, animal, and insect subjects using nanoCurie doses. Data for risk analyses of agricultural or industrial chemicals are obtained at environmentally relevant levels down to pg/kg doses by dermal, oral, or respiral exposures. Natural chemical components in soils and waters are traced by AMS using the "bomb spike" of radioisotopes from the 1960's. Labeled compounds and their derivatives can be quantified in 100 μl of sampled soil (assuming an organic carbon content of 5 - 10 g/l) after releases of <<50nCi/m3. All these exposures leave the samples and wastes below the 50 nCi/g definition of "radioactive" or "mixed" wastes, minimizing costly disposal. The principles of AMS are described and examples are drawn from dermal exposures of chlorinated hydrocarbons and organophosphates, oral exposures of benzene and permethrin, and human consumption of labeled nutrients.

# DEVELOPMENT OF ENZYME-LINKED IMMUNOSORBENT ASSAYS (ELISA) FOR ESFENVALERATE METABOLITES IN HUMAN URINE

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The pyrethroids are one of the most heavily used insecticide classes in the world. It is important and significant to develop sensitive and rapid analytical techniques for assessments of human exposure to these compounds. Highly sensitive and selective ELISAs for glycine conjugates of key esfenvalerate metabolites, phenoxybenzoic acid (PBA) and fenvaleric acid (FA) were developed. Rabbits were immunized with either N-(3-phenoxybenzoyl) 4-amino-L-phenylalanine-Fetuin or N-[(S)-4-chloro-2-(methylethyl) benzeneacetyl]-4-amino-L-phenylalanine-Fetuin, and all sera were screened against numerous coating antigens. The assays with the least interference and best sensitivity were optimized and characterized. The IC<sub>50</sub>s for PBA-glycine and FA-glycine in buffer are  $0.42 \pm 0.18$  and  $0.40 \pm 0.12$  µg/L, respectively. Both assays exhibited high selectivity. No or little cross reactivity was measured to the parent compound and other Urine matrix effect was investigated. Solid phase extraction (SPE) strategies were also developed in an attempt to increase the sensitivity of the overall method. The lower detection limits (LDL) for PBA-glycine and FA-glycine in urine with SPE are 0.72 and 0.60 µg/L, respectively. These assays could be used as markers of exposure for monitoring biological samples.

# DEVELOPMENT OF AN IMMUNOASSAY FOR THE PYRETHROID INSECTICIDE ESFENVALERATE

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A competitive enzyme-linked immunosorbent assay (ELISA) was developed for the detection of the pyrethroid insecticide esfenvalerate. Two haptens containing amine or propanoic acid groups on the terminal aromatic ring of fenvalerate molecule were synthesized and coupled to carrier protein as immunogens. Five antisera were produced and screened against eight different coating antigens for titration. The assay with least interference and best sensitivity for esfenvalerate was optimized and characterized. This assay is a homologous system with antibody 7588 and coating antigen 4-[3-[Cyano-[(S)-2-(4-chlorophenyl)-3-methyl-1-oxobutanoxy]methyl]] phenoxy] benzenepropanoic acid (7)-BSA. The IC<sub>50</sub> for esfenvalerate was 30  $\pm$  6.2  $\mu$ g/L and the lower detection limit (LDL) was 3.0  $\pm$  1.8  $\mu$ g/L. The selectivity of this assay is excellent. Other pyrethroid analogues and esfenvalerate metabolites tested did not cross react significantly in this assay. In order to increase the sensitivity of the over all method, a C<sub>18</sub> sorbent-based solid-phase extraction (SPE) was developed for natural water samples with 85.9  $\pm$  3.9 % recovery rate for <sup>14</sup>C-labelled fenvalerate. With this SPE step, the LDL of this assay for esfenvalerate is 0.1  $\mu$ g/L in water samples.

# DEVELOPMENT OF AN ENZYME IMMUNOASSAY FOR 4-NITROPHENYL-GLUCURONIDE

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Immunoassays can be used to determine the concentrations of xenobiotics and/or their metabolites in the tissues and body fluids of exposed populations. They offer the advantages of a reduced need for sample clean-up, very rapid through-put of large sample sets, and a low cost per sample compared to classical analytical methods, such as GC and HPLC. As one of the primary human urinary metabolites of the highly toxic insecticides methyl and ethyl parathion, 4-nitrophenyl-glucuronide (4NPG) is an excellent target for such immunoassay development. Our goal was to develop an enzyme immunoassay for 4NPG that can be used for biomonitoring of methyl and ethyl parathion exposure in humans. 4NPG was conjugated to hemocyanin and to other proteins, and coupling was confirmed. Rabbits were immunized with 4NPG-hemocyanin to generate polyclonal antibodies. Other conjugates were synthesized for use in homologous and heterologous competitive immunoassays. High titers and good selectivity were observed in the sera from two of the three rabbits. Using standard procedures, an enzyme immunoassay was developed and optimized. The best overall immunoassay, using a homologous coating antigen, has an IC50 in the low part-per-billion (ng/mL) range and a lower limit of detection below 1.0 part-per-billion. Assay selectivity is very high with the tested potential urinary cross-reactants showing less than 0.1% cross-reactivity. Spike recovery studies using human urine without any clean-up, demonstrate very good recovery. Polyclonal antibodies selective for 4NPG have been generated and an enzyme immunoassay, with utility in exposure analysis, has been produced.

# DEVELOPMENT OF A GROUP-SPECIFIC ENZYME IMMUNOASSAY FOR URINARY PHENOLIC GLUCURONIDES

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Phenol and related compounds are toxic agents to which humans are exposed. Because serological methods are suitable for the rapid and inexpensive monitoring of conjugated metabolites in human fluids, an immunoassay for glucuronide-bound phenolic compounds was developed. For that purpose, p-aminophenyl-glucuronide was coupled to the carrier protein having the glucuronic acid exposed. Rabbits were injected with the immunogen in order to rise polyclonal antibodies. The resulting immunoassay shows an inhibition by phenyl-glucuronide, p-nitrophenyl-glucuronide and p-aminophenyl-glucuronide in the low ppb (ng/ml) range. No cross-reactivities were observed for glucuronic acid, D-glucose, p-nitrophenol, p-aminophenol, phenol and p-nitrophenyl-galacturonide. Thus the assay can be used to assess human exposure to complex mixtures of xenobiotics, such as phenol, benzene, nitrophenol and phenylamines known to be excreted in urine as phenolic glucuronic acid conjugates.

#### A NEW TAPE STRIPPING ASSAY FOR DETECTION OF METALS IN SKIN

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Occupational and environmental exposure to metals and metal compounds raises concerns about their effects on human health. A simple minimally invasive method to detect metals on and in skin would be a valuable assessment tool for quantifying both topical exposure and the effectiveness of skin cleanup measures. We have developed a novel low-metal adhesive tape for this purpose using commercially available components (adhesive, backing and release liner). The low-metal tape is applied to the skin surface and then removed, taking with it a sample of the dead outer layer of the skin (the stratum corneum, or SC). These tape-strip samples are then analyzed by particle-induced X-ray emission (PIXE), a well-characterized analytical technique based on X-ray spectrometry, which identifies and quantitates metals with ppm sensitivity and provides twodimensional spatial resolution as well. The feasibility of the assay was demonstrated using human volunteers. The tape-strip samples were standardized by trans-epidermal water loss (TEWL) measurements of the skin in vivo, tape weight, and microscopic examination of the tape. Method utility tests demonstrated consistency within a single sample, within a subject over eight consecutive days, and between subjects. SC sample weights decreased with successive tape strips at the same location, and a plot of 1/TEWL versus weight had r2=0.9196. In summary, a quantitative, minimally invasive tapestriping assay for the detection of metals on and in skin (and other surfaces) has been devised, and its utility has been demonstrated. This new technique provides depth information not available from wipe or swab sampling of skin.

# MOUNT SINAI SUPERFUND ENVIRONMENTAL HEALTH COMMUNITY AND SCHOOL OUTREACH PROGRAM, LEAD AND ORGANOCHLORIDES SUPERFUND OUTREACH

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This poster will feature the community outreach and environmental health science aspects of a Superfund Research Project of the Mount Sinai Medical Center's Superfund Program in New York City. The Poster will depict elements of a two-pronged program: one for the community living in neighborhoods particularly impacted by lead and organochlorides, increasing awareness and learning about the science behind the environmental health concerns in the City; the second prong of Mount Sinai's outreach is a collaboration with a public school, the High School for Environmental Studies, where we have developed a brand-new environmental health curriculum that is being adopted as an elective science course at the school. This new environmental health curriculum was developed in collaboration with teachers from that high school, their students, and a host of Superfund Investigators at Mount Sinai Medical Center. about 135 HSES students have taken this year-long course. They conducted research, prepared newsletters to the community, and studied the scientific literature - especially the latest reports from Superfund NIEHS/EPA. Some of them prepared Westinghouse papers for which they received academic credit through the high school course they took at Mount Sinai (taught by their teacher who was assigned to work on this project).

#### THE EFFECT OF SKIN CONTACT ON DERMAL ABSORPTION FROM SOIL.

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Skin-soil contact and contact frequency are important determinants of exposure to soil contaminants yet these factors are not accounted for in current dermal exposure assessment guidelines. Experiments were conducted to evaluate the effect of skin-soil contact and contact frequency on the dermal absorption of <sup>14</sup>C-pentachlorophenol (PCP) from soil. Soil loads ranging from 1 to 10 mg/cm<sup>2</sup> at a <sup>14</sup>C-PCP concentration of 200 ng/g were applied to human skin in vitro for periods up to 24 hr with two methods. Soil was applied by either pressing soil into the skin with a force of 0.65 psi or simply depositing soil on top of the skin surface. Soil was applied as multiple doses or as a single dose with both methods. 14C-PCP retained in the skin was determined with accelerator mass spectrometry. Skin uptakes from multiple applications of soil applied with the two methods were significantly different, while uptakes from single applications using the two methods were not. Uptakes from multiple soil doses applied with the press and deposition methods, normalized for soil load, were 6.1  $\pm$  6.4 and 1.7  $\pm$  0.7 fmol <sup>14</sup>C/cm<sup>2</sup>, respectively. The percentage of the dose absorbed from multiple applications with the deposition method was inversely related to soil loading, whereas no change in the absorbed dose was observed with increasing soil loads applied with the press method.

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# CORRELATING BACTERIAL TRANSPORT IN POROUS MEDIA WITH ADHESION FORCES

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Improving the transport of bacteria in porous media may be necessary for bioremediation through bioaugmentation to be successful. Surface modifying chemicals (Tween 20, lysozyme, EDTA, proteinase K, penicillin, sodium pyrophosphate, disodium tetraborate) have been shown to alter the attachment of bacteria to soil grain surfaces. The silicon tip of an atomic force microscope (AFM) is very hydrophobic, and bacteria that are attracted to the tip will not be attracted to soil, which is hydrophilic. It was hypothesized that increased adhesion between bacteria and a surface as a result of surface modifying chemicals could be measured as a reduction in the adhesion force between the bacteria and the hydrophobic AFM tip. An AFM was therefore used both to image the topography of bacteria surfaces and to develop force-versus-distance curves between the tip and the cell surfaces. Bacteria were prepared for AFM imaging and force measurements by cross-linking carboxyl groups on the cell surfaces to aminosilane compounds that had been attached to glass slides. Images and force measurements were conducted in air using tapping mode, so as not to damage the cells. Exposing Burkholderia cepacia (G4) and Pseudomonas stutzeri (KC) to surface modifying chemicals changed the roughness of the cell surfaces and the adhesion forces between the bacterium and the silicon tip. Both of these bacterial strains are of interest in bioremediation because under appropriate conditions G4 can degrade trichloroethylene and KC can degrade carbon tetrachloride. The changes in measured adhesion forces were correlated to transport measurements made using radiolabeled cells in soil mini-columns. This AFM technique may provide a more cell-specific method than currently available for quantifying adhesion forces between cells, and structures on cell surfaces, with soil grains and other surfaces covered with surface-modifying chemicals.

#### INVESTIGATION OF MOTILE MICROBIAL MIGRATION IN POROUS MEDIA

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Knowledge of subsurface bacterial migration is crucial for the design of aquifer bioremediation, bioaugmentation, and for describing bacterial movement within contaminant plumes. The extent of microbial migration through porous media is largely determined by deposition rates. Studies were conducted to quantify two transport parameters; collision efficiency (a) and an effective diffusivity coefficient (D<sub>eff</sub>). In order to estimate the extent of bacterial migration, it was sought to determine a correlation between α and D<sub>eff</sub>. These parameters were used to characterize bacterial transport in saturated heterogeneous porous media for Pseudomonas sp. Strain B13 a 3chlorobenzoate degrading motile organism. The collision efficiency  $\alpha$ , defined as the fraction of cells colliding with the soil grain surface resulting in attachment, was experimentally identified using intracolumn deposition data from 7-cm porous media columns inoculated with radiolabeled cells suspended in low ionic strength solution. Using motility assays, a random diffusivity coefficient D<sub>R</sub> was determined which accounts for cell transport in the bulk aqueous phase. From D<sub>R</sub>, an effective diffusivity coefficient  $D_{eff}$  can then be determined according to  $D_{eff} = (\epsilon/\tau)D_R$  where  $\epsilon$ =porosity and τ=tortuosity. It was hypothesized that a high random diffusivity coefficient, or high D<sub>eff</sub>, will result in extensive bacterial transport (low  $\alpha$ ) while a low random diffusivity coefficient, or low  $D_{eff}$  will result in relatively low bacterial transport (high  $\alpha$ ). Furthermore, deposition data obtained under unfavorable (presence of interparticle repulsion) conditions for bacterial density profiles at three different flow regimes were examined to determine an effect of previously deposited cells on the transport for this motile species.

### CONCENTRATION DEPENDENT SURFACE TENSION FOR AQUEOUS SOLUTIONS OF ALKYL SUBSTITUTED BENZENES

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Surface tension affects a number of processes important to the transport and fate of organic contaminants, and to remediation schemes for restoring soils and groundwater. For instance, pressure head in unsaturated porous media is proportional to surface tension, and consequently variations in surface tension influence the unsaturated flow of water. Additionally, surface tension highly affects the efficiency of remediation techniques that depend on bubble size and rise velocity (e.g., air stripping and air sparging). It is well understood that the surface tension of water decreases with addition of individual hydrophobic or surface active solutes. However, quantitative relationships between surface tension and concentration are generally unknown for aqueous solutions containing single and multiple dissolved compounds of environmental concern. Determination of the surface tension of aqueous solutions is necessary for improved estimation and modeling of contaminant fate and transport in environmental systems and for design optimization of associated remediation technologies.

Axisymmetric Drop Shape Analysis (ADSA) with digital image processing was employed to determine isothermal relationships between equilibrium surface tension and concentration for aqueous solutions of benzene and alkyl substituted benzenes. Experiments were conducted by contacting advecting air streams containing water and organic solute vapor with water pendant drops in an inert environmental chamber. Systems were analyzed in duplicate at five temperatures of environmental interest (285.2 K, 291.2 K, 297.2 K, 303.2 K, and 315.2 K), for solute concentrations up to saturated vapor pressure. Surface tension measurements of single-solute aqueous solutions have similar isothermal trends, with reductions in surface tension by as much as twenty percent. Measurements of solutions with multiple solutes show complex and non-ideal variations in surface tension with species concentration.

### REDUCTIVE DISSOLUTION OF PYROLUSITE BY FE(II): A REAL-TIME IN SITU X-RAY ABSORPTION SPECTROSCOPY EXPERIMENT

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Early mining practices have left ground-water aquifers and surface-water bodies contaminated with heavy metals. A comprehensive understanding of the processes that control heavy metal transport is vital to protect water supplies. Numerous reactions occur along the flow path that alter the water chemistry, including precipitation (removal of metals) and dissolution (release of metals) of mineral phases. An understanding of molecular- and atomic-level processes that control precipitation and dissolution leads to more accurate field-scale reactive transport models.

One system of importance for mining-impacted sites is the reductive dissolution of pyrolusite (Mn(IV) oxide) by Fe(II), as transport of heavy metals is often dominated by iron and manganese chemistry. Results from batch experiments indicate a complex reaction: an initial rapid release of Mn(II), then a rate decrease of four orders of magnitude. Questions are: 1) is Mn(III) formed during the reaction, and if so, 2) does it control the release of Mn(II)? A flow-through reaction cell for real-time, in situ X-ray absorption spectroscopy (XAS) studies was developed to couple rate measurements with direct spectroscopic observation of element oxidation state.

Using the reaction cell, the XAS spectra were recorded during the reductive dissolution of pyrolusite by Fe(II) under acidic conditions. Fitting the XAS spectra with pyrolusite and Mn(II) solution spectra, resulted in a residual peak approximately halfway between the Mn(II) and Mn(IV) peaks, indicating the possibility of an Mn(III) species forming in the cell. Adding bixbyite (Mn(III) oxide) to the calculations significantly improved agreement with the data. In addition, the Mn(II) concentration tracked the Mn(III) concentration, indicating that the reduction of Mn(III) is the rate controlling step from Mn(IV) to Mn(II).

# REPRESENTATIVE PLANT AND ALGAL UPTAKE OF METALS NEAR GLOBE, ARIZONA

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Past acid-mining activities in the Globe-Miami, AZ area have resulted in the release of metal contaminants into the perennial reach of Pinal Creek. Manganese is the dominant metal species, but concentrations of trace heavy metals (e.g., zinc, nickel, and cobalt) are also increased compared to natural levels found in other nearby stream systems.

Our research has focused on identifying and quantifying key processes responsible for the observed removal of manganese and other trace metals from Pinal Creek. Trace levels of metals are necessary to aquatic plants and algae, but elevated concentrations can trigger inherent detoxification mechanisms resulting in bioaccumulation of excess metals. In order to determine whether this process was also occurring at Pinal Creek, and its potential significance in the observed natural, but limited, remediation of metals, we collected water speedwell (Veronica anagallis-aquatica), rabbitfoot grass (Polypogon monspeliensis (L.) Desf.), duckweed (lemna minor), and algae and moss.

Results indicate that bioaccumulation of manganese, nickel, cobalt, copper, zinc, and iron is occurring in Pinal Creek with apparent bioconcentration factors of 100 to over 10,000. Limited comparisons of uptake by plants in the nearby, but uncontaminated, Pinto Creek, indicate that, although the bioconcentration factors are similar, the mass of metals present in the aquatic plants at Pinal Creek is significantly higher, i.e., bioaccumulation is a function of the absolute metals concentrations.

Natural remediation of manganese and other metals by different aquatic plants and algae at Pinal Creek is occurring. The contribution to the total metals removal from Pinal Creek and the factors that limit the effectiveness of this process will be addressed in future field and laboratory work.

# DEMONSTRATION OF GAS-PHASE PARTITIONING TRACERS FOR THE IN SITU MEASUREMENT OF SOIL WATER CONTENT IN A LARGE WEIGHING LYSIMETER

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Characterizing water flow and solute transport in the vadose zone is important for the management of agricultural resources and for pollution prevention. Water content has a profound effect on the fundamental properties governing flow and transport behavior in soil. The majority of the measurement methods in current use provide what can be considered as point values of water content due to the relatively small sample volumes (10<sup>-1</sup> m scale). While this is an advantage for obtaining accurate information at small scales, it is a disadvantage for determining water contents for larger (field) scales. A large number of samples must be taken to fully characterize the system, especially in heterogeneous systems. The gas-phase partitioning tracer test provides a global value of soil water content with little prior knowledge of a system. The method is based on introducing a gas-phase partitioning tracer into the system. The partitioning tracer dissolves into the water, which retards its movement with respect to a non-partitioning This retardation is a function of the soil water content. partitioning tracer experiments are being conducted in a large weighing lysimeter at The University of Arizona Karsten Turf Center for Research. Water content values estimated from the retardation of the gas-phase partitioning tracer were close to gravimetric and time domain reflectometry (TDR) measurements, indicating that the tracer method has the potential to provide accurate values of water content at the field scale.

# ENHANCED REMOVAL OF AGED AND IMMISCIBLE PHASE TCE FROM AQUIFER MATERIAL

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The most common method for aquifer remediation is pump-and-treat. This method works well initially, removing large amounts of pollutant, however its effectiveness usually declines over the period of a few years due to chemical and physical processes within the aquifer. Techniques designed to increase pump-and-treat efficiency have been proposed including the addition of chemicals such as surfactants, cosolvents, dissolved organic matter, and complexing agents that act to increase the contaminant's apparent solubility.

One of the processes responsible for the difficulty in removing contaminants is slow desorption, in which contaminant aging plays a role. It has been observed in the lab and in field studies that the longer the contaminant has been in contact with the aquifer, the more difficult it is to extract. Another process responsible for the constrained removal is the presence of immiscible phases, which act as long term sources. The objective of this research was to determine the effectiveness of selected solubility-enhancing agents for removing TCE from systems influenced by contaminant aging or the presence of residual phases. Column studies were conducted using aquifer material from a Superfund site. The aged material was first immersed inside an aqueous-saturated TCE solution for 2 years before being packed into separate columns. The residual phase was introduced into a separate set of columns of unaged aquifer material. Enhanced removal rates were compared within each column set and between the aged and residual sets. These results were also compared to those obtained using unaged aquifer material with no residual phase.

# THE IMPACT OF BACTERIAL CELL GROWTH AND MICROBIAL LAG ON THE TRANSPORT AND BIODEGRADATION OF ORGANIC COMPOUNDS

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Miscible-displacement experiments were conducted to examine the impact of microbial variables (such as cell growth and metabolic lag) on the biodegradation and transport of salicylate, a model hydrocarbon compound. For each experiment, a soil column was inoculated with bacteria that were genetically engineered to degrade naphthalene and salicylate, and then subjected to a step input of salicylate solution. Oxygen availability, cell growth, and microbial lag were examined to determine their effect on the characteristic shape of the salicylate breakthrough curve. For all cases examined, the transport behavior of salicylate was nonsteady, and an overall mass loss was observed. The total amount of salicylate that was degraded in the column was increased by sparging the influent solution with oxygen, but it did not influence the shape of the initial breakthrough behavior of salicyate. The effect of microbial lag on the shape of the salicylate breakthrough curve was examined by exposing the column to two successive pulses of salicylate, thereby allowing the organisms to acclimate to the carbon source. Degradation of salicylate in the second pulse was immediate, compared to a delay in degradation of salicylate in the first pulse. The cause of the lag was further investigated using succinate, a compound that is constitutively degraded (no metabolic lag). A very slight lag effect, attributed to physiological perturbation, was observed in the succinate breakthrough curve. Therefore, it was determined that a metabolic microbial lag, rather than a physiological lag, is the primary behavior observed in the characteristic nonsteady transport behavior of salicylate. Elimination of microbial lag effects allowed the impact of bacterial growth on salicylate breakthrough to be quantified.

#### **PERCHLORATE**

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Perchlorate (ClO4) has been discovered in groundwater and Colorado River in CA, NV and AZ - affecting the drinking water, agricultural and recreational supplies for over 15 million people in three states and possibly 11 tribes. The ammonium and potassium salts of the perchlorate anion are primarily used as a component of solid rocket fuel, and perchlorate is notably soluble, stabile and highly mobile in aqueous systems. Major questions remain on the toxicology, analytical methodology and treatment technology of this contaminant. At high levels, it is known to disrupt thyroid function in humans and animals. Perchlorate has an impact on Superfund cleanups, RCRA sites, drinking water programs, state and tribal relations, children's health issues, federal facilities and national response and policy.

# COUPLED TRANSPORT AND BIODEGRADATION OF VOLATILE ORGANIC COMPOUNDS IN UNSATURATED SOIL

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Coupled vapor phase chemical transport, sorption, dissolution, and biodegradation were studied in soil columns. Two volatile organic compounds were used; toluene and trichloroethylene (TCE). Toluene degradation occurred readily, whereas TCE degradation only occurred in the presence of toluene. Furthermore, TCE exerted competitive inhibition effects on toluene degradation when the vapor concentration ratio of the two compounds approached unity. Diffusive transport was described by Fick's law, and sorption and dissolution were both described by linear, equilibrium functions. Toluene degradation was modeled by describing the kinetics of growth and decay of the microbial populations. Trichloroethylene degradation was modeled as a linear fraction of the rate of toluene degradation. All parameters needed in the model were obtained from batch and sterile column experiments, except for the initial biomass, which was fitted to the collected data. In the absence of a biodegradation lag period, a model combining the different processes was able to predict the spatial and temporal distribution of the chemical concentrations in soil column experiments. At regions where extensive toluene degradation occurred, microbial growth was limited by depletion of soil nitrogen. Complete degradation of TCE was never attained due to the depletion of toluene from the system. For cases of biodegradation lag periods, both compounds moved through the soil column during the lag phase, then diffused back into the soil column when biodegradation started. Lag periods were readily modeled using biomass growth parameters without incorporating enzyme induction processes. In general, parameters obtained from independent experiments describing toluene and TCE sorption, diffusion, dissolution, and biodegradation kinetics were successfully used to describe the fate of these compounds during coupled transport and biodegradation in soil.

#### DETERMINATION OF MONOD PARAMETERS AND TRICHLOROETHYLENE TRANSFORMATION CAPACITY FOR METHYLOSINUS TRICHOSPORIUM OB3B MUTANT PP358

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The monod parameters for Methylosinus trichosporium OB3b (mutant strain PP358) were determined for the uptake of methane and trichloroethylene (TCE) using a biooxygen monitoring system. Unreliability that is derived due to mass transfer across the gas-liquid interface was avoided by eliminating the reactor space. Maximum methane and TCE oxidation rates were 75.6 and 23.4 nmol/min/ mg/l CDW, respectively. Half-saturation constants for methane and TCE were 65.4 and 30.1(M. Another important parameter that bears on the feasibility of microbial TCE degradation, the transformation capacity (Tc), was also established for the mutant species. Previously Tc was defined as the maximum amount of TCE transformed per active cell mass prior to loss of TCE transformation activity (Tc=0.118mgTCE/mg cell). Here, transformation capacity was measured semicontinuously to determine whether cell inactivation was proportional to TCE transformation through the cause of an incubation. The cell inactivation was monitored by the sMMO activity in the cells. The transformation capacity is redefined as the ratio of TCE degradation and sMMO activity change. Our study shows that the rate of sMMO loss from cells is not proportional to the TCE mass transformed. The transformation capacity had the maximum at the beginning, and decrease along with the increase of TCE oxidized.

#### IMPACT OF EXPOSURE TO GOLD MINING HAZARDOUS WASTE ON WILDLIFE

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In excess of 100 million pounds of sodium cyanide is currently used annually in Nevada in the cyanidation process for the extraction of gold and silver. The waste solutions from this processing form large ponds near the mining operation which may reach sizes in excess of 500 acres. These ponds pose a significant risk to surrounding wildlife as well as migratory wildlife and can contaminate ground and surface water resources when leaks or flooding of these impoundments occur. There are currently no state or federal regulations concerning acceptable cyanide levels in these waste impoundments. The current industry-wide held belief, based on anecdotal evidence, is that maintenance of these ponds at or below 50 ppm WAD cyanide is safe.

A biochemical assessment was conducted, using mallard ducks as a migratory bird model, on the impact of exposure to various levels of cyanide around the 50 ppm level. Studies were conducted to assess the bioenergetic effects of cyanide exposure on these birds. The results of these studies show that exposure to cyanide in the birds drinking water at levels less than 50 ppm, while not acutely toxic, did cause significant mitochondrial dysfunction as well as a reduction in ATP levels in heart, liver and brain tissues. The biological implications of these bioenergetic effects are being assessed using a homing pigeon model developed to assess xenobiotic impacts on bird flight. The preliminary results from these studies suggest a significant impact on flight endurance following cyanide exposure. These studies were supported in part by a grant from the NIEHS Superfund Basic Research Program (1 P42 ESO5961) and the Nevada Agricultural Experiment Station.

#### REACTION OF MERCURY CYANIDE (HgCN2) IN PRECIOUS MINING FLUIDS

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Mercury cyanide complexes, primarily dicyanomercury Hg(CN)2, are formed as by-products during extraction of precious metals from mercury-containing ore using cyanide solutions. Unlike divalent mercury, Hg(CN)2 is highly water-soluble (0.4M) at neutral or basic pH, and can potentially be delivered to surface and groundwater systems when precious metal fluids are released to the environment. This study was designed to examine the environmental reactions of Hg(CN)2. In our studies, a new liquid chromatographic method was developed for the determination of Hg(CN)2 in water without decomposition of complexes. A Porous Graphitic Carbon (PGC) column coupled with a UV-VIS detector was used, and a detection limit of ~2 mg/L was obtained. Using this new method, several chemical and photochemical properties of Hg(CN)2 were investigated. Although Hg(CN)2 in unionized in water, its 1-octanol/water partition coefficient (Kow) at pH 4-7 is small (<10-3). Dicyanomercury was found to be stable over a wide pH range of 3.6-8.0. The release of mercury ion (Hg2+) from Hg(CN)2 increases when samples are strongly acidified with H2SO4 or HCI. At neutral and/or high pH, the addition of OH-, SO42-, and CI- results in the conversion of Hg(CN)2 to other complexes. High concentration of free cyanide (<10-3 M) at pH>9 results in the formation of tri-, or tetracyanomercury (Hg(CN)3-, or Hg(CN)42-). Hg(CN)2 was found to be photochemically stable under xenon arc irradiation.

# IMPORTANCE OF MURINE CYP2F2 IN THE METABOLIC ACTIVATION OF NAPHTHALENE AND IN THE METABOLISM OF OTHER XENOBIOTICS

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Although the levels of pulmonary cytochrome P450 monooxygenases are substantially lower than those in the liver, these enzymes play an important role in the metabolic activation of substrates which cause lung injury. The target and species selective toxicity of a number of pulmonary injurants has been attributed to localization of enzyme and to the presence of enzymes with high Kcat in target airways of susceptible species. However, experimental demonstration of these concepts and quantitative assessment of the contribution of individual P450 isoforms is lacking. Naphthalene is an environmental contaminant which causes selective toxicity to murine Clara cells at low doses. Naphthalene-induced Clara cell toxicity correlates with the presence of Cyp 2f2, the putative enzyme responsible for the stereospecific metabolism of naphthalene to 1R,2S naphthalene oxide. The finding of an orthologous enzyme in human lung (CYP2F1) supports the need to understand the metabolic and kinetic differences in the activation of lung toxicants between species. To further correlate findings in animal models to human toxicity. By understanding the mechanism by which a toxicant is metabolized, biomarkers of exposure can be found.

This study was done to characterize the catalytic activities of Cyp2f2 with as well as other xenobiotics that either undergo metabolic activation to cytotoxic intermediates or that are 'isoform selective' substrates. Cyp2f2 was generated using a baculovirus expression system. Incubations of Tn5 cell lysate containing recombinant Cyp2f2 with NADPH-cytochrome P450 reductase and NADPH metabolized naphthalene with a high degree of stereoselectivity to the 1R,2S-naphthalene oxide (66:1 enantiomer ratio). Km and kcat values along with the specificity constant for naphthalene metabolism by Cyp2f2 were 4.7 µM, 118 min-1, and 4.2x105 M-1sec-1, respectively. Cyp2f2 also metabolized ethoxyresorufin, pentoxyresorufin, p-nitrophenol, 4-ipomeanol and '1-nitronaphthalene at easily detectable levels. These studies suggest that Cyp2f2: 1) plays a key role in the species and cell selective toxicity of naphthalene, and 2) effeciently metabolizes a number of other substrates including some thought to be isoform selective. Supported by NIEHS 04311, 05707, ES04699.

### AN ECOLOGICAL SURVEY OF BIOSURFACTANT-PRODUCING MICROORGANISMS IN ARID SOUTHWESTERN SOILS

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Biosurfactants are a unique class of compounds that have been shown to have a variety of potential applications including; enhanced remediation of organic- and metalcontaminated sites, enhanced transport of bacteria, enhanced oil recovery, cosmetic additives, and biological control. However, little is known about the distribution of biosurfactant-producing microorganisms in the environment. The goal of this study was to examine the ecology of surfactant-producing organisms in order to determine how commonly they occur in pristine and contaminated sites. A series of contaminated (metals or hydrocarbons) and uncontaminated soils were collected. Soils were plated on R2A agar and each unique isolate was grown in mineral salts medium containing 2% glucose to screen for biosurfactant production. Supernatants from each culture were tested using the drop-collapse method to determine their ability to reduce surface tension of a solution. Microorganisms classified as positive for biosurfactant production from the drop collapse test were then grouped by ERIC analysis of their genomic DNA. For each unique group of biosurfactant-producers, 16S rDNA sequencing was performed to identify the isolates. A total of 32 biosurfactant-producing isolates have been obtained thus far, two of which have been identified as Pseudomonas and Bacillus. Of the 32 isolates, the majority were gram positive and were obtained from heavy metalcontaminated soils (cadmium and/or lead). Overall, these results indicate that biosurfactant-producing organisms are found in most soils suggesting that in situ production of biosurfactants in contaminated sites may be feasible.

### BIOSURFACTANT-INDUCED PROTECTION AGAINST CADMIUM TOXICITY DURING PHENANTHRENE DEGRADATION IN SOIL

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The presence of heavy metals can inhibit the degradation of organic compounds in sites co-contaminated with metals and organics. Previous work in our laboratory has shown that a rhamnolipid biosurfactant produced by Pseudomonas aeruginosa can complex metals commonly found in contaminated sites such as cadmium and lead. It has also been demonstrated in pure culture studies that rhamnolipid can mitigate cadmium toxicity during biodegradation of naphthalene. The goal of this study was to investigate whether rhamnolipid could mitigate the toxic effects of a model metal, cadmium, on an indigenous soil microbial community during the degradation of phenanthrene. The soil chosen for this study was a Brazito sandy loam which has an indigenous population of phenanthrene degraders. Cadmium (390 mg/kg) was added to the soil so that the final concentration of cadmium in solution, considered the bioavailable concentration, was 3 This concentration of cadmium was found to inhibit phenanthrene (500 mg/kg)degradation for at least 1 week. The effect of rhamnolipid on phenanthrene degradation in cadmium-contaminated soil was then evaluated for rhamnolipid concentrations of 100, 1000, 10,000 mg/kg. Results indicate that rhamnolipid added at 1000 mg/kg protected against cadmium toxicity. Rhamnolipid added at 100 mg/kg had no effect and rhamnolipid added at 10,000 mg/kg was inhibitory to the indigenous These results suggest that rhamnolipid may be useful in enhancing bioremediation of organic contaminants in sites that are co-contaminated with organics and metals.

#### ACTIN AS A TARGET FOR REACTIVE METABOLITES OF NAPHTHALENE

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There are significant uncertainties in estimating the risks to humans of exposure to compounds from either environmental or occupational sources. Extrapolation to humans of toxicology data obtained in animals remains a significant source of that uncertainty. There are several methods that may be utilized to aid in the determination of the importance of environmental or occupational exposures in human toxicology and these include the development, validation and application of biomarkers to human populations. We argue that these will provide the most value if the biomarker is closely tied to the mechanisms by which the agents in question produce toxicity. Naphthalene elicits a very species, tissue and cell selective lesion of the murine Clara cell. Detailed time course studies in target airways have shown that this lesion is preceded by extensive glutathione depletion and by the formation of covalent adducts. Recent work has identified actin as one of the protein targets by co-migration on one and two D gel electrophoresis, by peptide mapping and by Western immunoassay with anti-actin antibody. These findings are consistent with early changes in Clara cell ultrastructure including swelling of the apical cytoplasm, congregation of mitochondria in the supranuclear cytoplasm and eventually, by 3 hours postinjection, blebbing of the apex of the cell. Supported by NIEHS 04699, 06700, and 04311.

#### QUANTIFICATION OF TRANSPORT MECHANISMS OF PERCHLORATE BRINES

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Perchlorate is becoming an increasing concern in the drinking water of California and other southwestern states. This compound is particularly interesting because the provisional EPA MCL is 18 ppb, but environmental concentrations can realistically reach 200,000 ppb. Perchlorate is released from manufacturing facilities and testing sites as ammonium perchlorate, a component of solid rocket fuel. Ammonium perchlorate is a highly soluble salt that, once wet, forms a dense brine in which the salt is completely dissociated. This brine is expected to migrate through the vadose zone and become a dense plume in the groundwater aquifer. An investigation of the transport behavior of this dense plume is key to the determination of the human health risk, and the development of an environmental remediation strategy.

Three mechanisms of brine transport are being investigated. The first of which is transport of the brine through an idealized aquifer. This analysis shows that, under steady state conditions, the ratio of the horizontal dimension of the plume to the vertical dimension is expected to be much greater than one. Also being considered is diffusion-controlled transport of perchlorate from a low permeability zone to a high permeability zone. Results show that even though significant concentrations can exist in the less permeable region, the amount of perchlorate loading is very small. Finally, an investigation of the fate of the perchlorate counterion, ammonium, is being preformed. Results show that the ammonium will be significantly retarded, and will displace all other exchangeable cations in the soil matrix. Analysis of the behavior of the ammonium and the soil cations can be developed into a diagnostic tool for predicting the history of the plume.

# MICROBIAL REBOUND IN SOIL AFTER STEAMING: ACTIVATION OF PAH DEGRADERS

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Steam enhanced extraction is an *in situ*, subsurface remediation technique that can effectively remove volatile and semi-volatile NAPL contaminants. Polynuclear aromatic hydrocarbons (PAHs), however, are less efficiently removed by the process. This research evaluates the effects of steam injection on soil microbial activity and the potential for in situ bioremediation of PAHs following steam treatment. A bench-top steam generator was used to steam three different soils: a laboratory prepared microbially-enriched soil, and two soils from petroleum-contaminated field sites.

Microbial activity was evaluated directly in the soil matrix during and after steam treatment using direct epifluorescent microscopy (DEM) assays with the respiratory activity dye CTC and the fluorochrome DTAF to quantify viable and total bacterial numbers, respectively. DEM results indicated that upon cooling, mesophilic bacterial activity rebounded to levels equivalent to those seen in unsteamed control soil.

The degradation potential of the microbial communities following steaming was also investigated in slurry cultures with various substrates including phenanthrene. These studies provided evidence that organisms capable of degrading PAHs were among the rebounding mesophilic cultures. Every soil tested showed positive growth on phenanthrene after steaming/in-place cooling. With contaminated soils, culturability on phenanthrene actually improved following steaming. Soil stored for four months following steaming showed the same slurry growth trends as freshly steamed soils, suggesting that the shift in microbial ecology is long term. A phenanthrene degrading organism (a *Sphingomonas* species) was isolated from a steamed soil. Oligonucleotide probes specific for this organism are being developed and will be used to track populations directly in the soil. Experiments are being designed to quantify post-steam PAH biodegradation and to determine whether the isolated organisms are important players in the degradation.

# AN EPIFLUORESCENT MICROSCOPY BASED METHOD FOR EVALUATING ANAEROBIC MICROBIAL ACTIVITY

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The use of 5-cyano-2,3,-ditolyl tetrazolium chloride (CTC) for the rapid quantitation of metabolic activity of aerobic microorganisms has gained wide application in ecological and environmental studies in recent years. CTC is a tetrazolium dye that is reduced to fluorescent intracellular formazan precipitates by components of the electron transport system and/or dehydrogenases of metabolically-active bacteria and can be visually detected by direct epifluorescent microscopy. Although it is well established that CTC can be used to detect aerobic microbial activity it was unclear whether this tetrazolium dye could be used to detect anaerobic microbial activity. Because of the potential for the reduction of some tetrazolium redox dyes by strong chemical reductants, their utility in assessing anaerobic microbial activity or microbial activity in low redox potential environments was generally thought to be doubtful. In this study we evaluated the utility of CTC in capturing anaerobic microbial activity and examined the factors contributing to abiotic reduction of CTC. We used CTC in conjunction with the fluorochrome stain 5 -(4,6-dichlorotriazinyl) aminofluorescein (DTAF) that targets bacterial cell wall proteins to quantitate total bacterial numbers as well as to determine the fraction thereof that is active. Diverse groups of anaerobic bacteria including fermentative bacteria, nitratereducing bacteria, iron-reducing bacteria, syntrophic bacteria, sulfate-reducing bacteria, and methanogenic bacteria effectively reduced CTC to intracellular CTC-formazan. CTC was abiotically reduced by ascorbic acid, cysteine hydrochloride, dithiothreitol, NADH, NADPH, Fe(II)Cl<sub>2</sub>, thioglycollate and sodium sulfide. The results of this study suggest that although CTC can be used to effectively quantitate the metabolic activity of anaerobic bacteria, care must be taken to avoid the abiotic reduction of CTC.

# SUBSTRATE INTERACTIONS DURING THE BIODEGRADATION OF BTEX AND FUEL OXYGENATE MIXTURES

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Petroleum hydrocarbons are common groundwater contaminants. Of particular concern are BTEX compounds (benzene, toluene, ethylbenzene, o-xylene, m-xylene and p-xylene), and fuel oxygenates such as MTBE (methyl tert-butyl ether) which has been noted for its persistence and mobility in aquatic systems. The specific goals of this research project are:

1. To evaluate the biodegradation and mineralization potential of BTEX mixtures by one mixed culture and two pure cultures, *Rhodococcus rhodochrous* and *R. bronchialis*, derived from a gasoline contaminated aquifer.

Our laboratory studies have revealed that all three cultures were capable of degrading each of the BTEX compounds, individually and in mixtures. Using  $^{14}$ C-labeled aromatics, the mixed culture was shown to mineralize all the BTEX components to  $^{14}$ CO<sub>2</sub>. Although the two pure isolates were able to biotransform o-xylene, they were unable to mineralize it. We have performed studies to determine the biochemical pathways employed by the pure cultures in mineralizing BTE(m-/p)X mixtures.

2. To identify and quantify substrate interactions during the biodegradation of BTEX mixtures.

Substrate interaction patterns included no interaction, stimulation, competitive inhibition, non-competitive inhibition and cometabolism. Ethylbenzene was shown to be the most potent inhibitor of BTX biodegradation by the three cultures.

3. To evaluate the biodegradability of MTBE and other fuel oxygenates, and to quantify their impact on BTEX bioattenuation.

MTBE was not biodegraded by the three cultures over a range of concentrations (2 to 200 mg/L). Furthermore, the presence of MTBE at concentrations of 2 to 150 mg/L in bi-substrate mixtures with each of the BTEX compounds had no negative effect on BTEX biodegradation rates. Ongoing studies are investigating the effects of ETBE, TAME, DIPE, methanol and ethanol on BTEX bioattenuation. Additionally, a propane-oxidizing consortium derived from a defunct landfill is currently being tested for the biodegradation of these oxygenates.

### SPERM DISOMY 21 IS ASSOCIATED WITH SEX CHROMOSOMAL ANEUPLOIDIES BUT DOES NOT PREFERENTIALLY SEGREGATE WITH THE Y CHROMOSOME: A STUDY OF 38 HEALTHY FERTILE MEN

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The purpose of this study was to determine whether (a) there is an association between the frequencies of disomy 21 and sex chromosomal aneuploidy in sperm of fertile men and (b) there is preferential segregation between disomy 21 and either sex chromosome. Sperm aneuploidy and diploidy were examined in semen samples from 38 healthy men of proven fertility (aged 20 - 50) using multi-color fluorescence in situ hybridization (FISH) with chromosome specific probes for X, Y and 21. All men had recently fathered a child with a sex chromosomal trisomy. Approximately 380,000 sperm were analyzed, ~ 10,000 cells per donor. Hybridization efficiencies were about 99.5% and sex ratios of normal sperm (X-21:Y-21) did not differ from 1:1. The frequency of sperm with disomy 21 was significantly correlated with the frequencies of total sex chromosome aneuploid sperm (X-X, X-Y, Y-Y, and sex nullisomy, p = 0.005). Significant correlations (p < less> 0.002) were obtained between frequency of disomy 21 and frequencies for disomy X (r = 0.52), X-Y (r = 0.50), and sex chromosome nulls (r = 0.58), but less so with disomy Y (r = 0.58) = 0.26, p <greater> 0.10). Age had no effect on these relationships. Among 288 disomy 21 sperm, 134 were Y-carrying and 154 were X-carrying, (p = 0.9). Thus, we found no preferential association between disomy 21 and Y carrying sperm. Our results support the hypothesis for a genome wide, rather than chromosome-specific mechanism controlling the production of aneuploid sperm. Our data also suggest that sex-ratio disturbance observed among trisomy 21 live borns is not due to a distorted ratio of Ybearing disomy 21 sperm. [Performed under the auspices of the U.S. DOE by LLNL under contract W-7405-ENG-48 with support by NIEHS Superfund grant P42ES04705]

# A NOVEL MULTI-COLOR FISH METHOD FOR HUMAN SPERM DEMONSTRATES THAT POSTMELOTIC CHROMOSOME BREAKS OCCUR MORE FREQUENTLY THAN DUPLICATIONS AND DELETIONS.

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Structural aberrations, a major type of paternally transmitted chromosomal damage, have been associated with developmental defects and genetic diseases. Using the hamster method, we and other laboratories reported high proportions of sperm carrying structural chromosomal defects including stable and unstable aberrations. efficient and sensitive method was needed for investigating genetic, endogenous and environmental risk factors for chromosomal aberrations in sperm. We report a novel multi-probe FISH strategy for human sperm that detects two major types of chromosomal aberrations, using chromosome 1 as the genomic target. Duplications and deletions of chromosome lp are detected using a pair of centromeric and telomeric probes and represent damage events that occur before or during male meiosis. A second pair of probes for the alpha and classical satellite regions of chromosome 1 detects post-meiotic breaks within this region. By using an additional centromeric probe for chromosome 8, this method also detects disomy frequencies for two autosomes and diploidy. Baseline frequencies for chromosomal defects were determined for ~40,000 sperm from healthy donors. Frequencies of sperm carrying duplications and deletions of lp were similar to each other ( $\sim$ 6/10<sup>4</sup> sperm) suggesting that the causative mechanism(s) was symmetrical. Comparing these FISH frequencies with those observed by the hamster method suggests that duplication and deletion events represent, in part, meiotic segregation products of chromosomal rearrangements. The mean frequency of sperm showing breakage within the lq classical satellite region was higher (17.5/10<sup>4</sup> sperm, p <0.01) which compares well with the frequency of breaks in the same region using the hamster method. This study provides critical initial validation for a new sperm FISH methodology for evaluating the etiology and risk factors of chromosomal aberrations in human sperm. [Work performed under the auspices of the US DOE by Lawrence Livermore Natl Lab, contract W-7405-ENG-48, and funding from NIEHS Superfund P42ES04705]

#### CELL-BASED BIOASSAY FOR ESTROGENIC/ANTIESTROGENIC CHEMICALS

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Many environmental toxicants can affect estrogen homeostasis of an organism, and this may occur by a variety of mechanisms. In an attempt to identify such compounds, and to examine the mechanism(s) by which this may occur, an expression vector has been constructed such that the firefly luciferase gene is under the inducible control of four estrogen-responsive DNA enhancer elements (EREs). This vector has been stably transfected into human ovarian carcinoma (BG-1) cells to produce a cell line capable of detecting 1 pM 17β-estradiol with the induction of luciferase. This bioassay is being utilized to examine the mechanism(s) by which aryl hydrocarbon receptor (AhR) ligands, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), disrupt the estrogen signaling pathway. Co-treatment of these cells with 1 nM TCDD results in a 30% - 60% decrease in estrogen inducibility, and this effect occurs between 12hr and 18hr after treatment. The inability of TCDD pre-treatment to further reduce the estrogen response, compared to simultaneous treatment, suggests that this antiestrogenic activity is not due to enhanced estrogen metabolism. In addition, the decreased response is not due to dioxin-responsive elements (DREs) since they are not present in our reporter plasmid. Our results indicate that TCDD can exert some of its inhibitory action on estrogen responsiveness in human ovarian cells relatively rapidly, and that this effect appears to occur in a manner distinct from that previously described. This work is supported by NIH grants ES07685 and ES04699 and a UC TSR&TP fellowship.

#### **EVALUATION OF MTBE AUTOMOTIVE COMBUSTION BYPRODUCTS**

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MTBE (methyl tertiary-butyl ether) is an oxygenate that comprises 11% of nearly all reformulated gasoline (RFG) in California; it is used in about 30% of gasoline nationwide. MTBE is used as a component of gasoline primarily for its contribution to reduced automotive carbon monoxide emissions. RFG is intended to decrease toxic emissions (especially benzene) from motor vehicles and to reduce ozone formation. During cold start or malfunctioning conditions, not all of the fuel undergoes complete combustion, producing significant amounts of pure MTBE and its combustion byproducts.

This study examines existing data of MTBE combustion byproducts from three types of studies: reaction of pure MTBE under atmospheric conditions; reaction of pure MTBE at elevated temperatures; and vehicle studies using reformulated gasoline containing MTBE, including both dynamometer tests and actual on-road measurements. Different predominant products are formed at atmospheric conditions than at elevated temperatures. Tert-butyl formate and formaldehyde are the major products formed under ambient conditions, while isobutene, methanol, and formaldehyde are the key reaction products formed at elevated temperatures. Laboratory studies indicate that catalysts are very effective in converting MTBE, and that no "surprise" compounds are formed over the catalyst. Vehicle and on-road studies with MTBE in gasoline confirm increased formaldehyde and unburned MTBE emissions from gasoline containing MTBE. In particular, the extensive Auto/Oil collaborative research program examined the effects of MTBE in gasoline on speciated hydrocarbon emissions using dynamometer tests for different vehicle technologies. This evaluation of laboratory and vehicle studies is used as a basis for comparison with experimental work-in-progress.

The experimental component of this study uses a combustion-driven flow reactor with post-flame injection to determine the key combustion byproducts of different MTBE fuel mixtures: pure (chemical grade) MTBE, refinery grade MTBE (containing other oxygenates and contaminants), and a California Phase 2 RFG containing MTBE. The formation of MTBE and reformulated fuel combustion byproducts are detected using Fourier Transform Infrared (FTIR) spectrographic analysis. The experimental reactor conditions are intended to simulate temperatures and fuel stoichiometry in an engine under "worst-case" operating conditions when the catalytic converter is not operating and complete combustion is not achieved, as in cases of cold engine starts or malfunctioning catalysts. This experimental work investigates the formation of MTBE byproduct compounds most likely to be formed from oxygenated fuels, particularly aldehydes and alcohols.

#### RECOMBINANT ANTIBODIES TO DIOXIN

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The methylotrophic yeast, *Pichia pastoris*, transformed with mouse immunoglobulin G (IgG) genes encoding the gamma and kappa chains from a hybridoma (DD1) secreting monoclonal antibody against dioxin (A. Recinos III and L. Stanker), produced a functionally assembled intact monoclonal antibody. A recombinant yeast expression vector (pPICZalpha B) with dual expression cassettes was constructed, with each cassette carrying the cDNA copy of either the IgG gamma or kappa gene, under the control of the methanol-inducible alcohol oxidase (AOX I) promoter, fused to the Saccharomyces cerevisiae alpha-mating factor signal sequence. The intactness of the inserts and the accuracy of the junction sequences were confirmed using the polymerase chain reaction (PCR). P. pastoris, transformed with these constructs, secreted functionally assembled intact recombinant antibody molecules into the medium. The antibody-specific mRNA synthesis in recombinant yeast was demonstrated by Northern blot analysis. Immunoblot and ELISA analyses of concentrated culture supernatants harvested on day 5 of induction revealed the presence of murine-specific IgG. Assay of the culture supernatants by ELISA showed specific binding activity to dioxin analogs (provided by D. Gilman, J. Sanborn, S. Gee and B. Hammock). The P. pastoris-based expression system thus promises to facilitate large-scale production of recombinant antibodies for environmental applications. This research was supported in part by grants from the National Institute of Environmental Health sciences, NIH, with funding provided by the US EPA (5 P42 ESO4699) and the UC Davis Center for Environmental Health sciences (ESO5707).

### SIMULATION OF MERCURY FATE AND TRANSPORT IN THE CARSON RIVER, NEVADA

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In 1991 the U.S. EPA designated the Carson River as part of a Superfund site due to its high level of mercury contamination. Water column total mercury concentrations have been observed as high as 61 ug/L by the USGS, while some fish have methyl-Hg concentrations four times over the human health limit for consumption (1 ug/g). Hg bank concentrations are extremely high (avg. = 64,242 ug/Kg) and more than 95% of the total Hg in the water column is associated with particles. The importance of bank erosion on the transport and fate of mercury in this system is therefore apparent. Using the U.S. EPA RIVMOD, WASP4 and MERC4 codes, this study has focused on program modifications that predict bank erosion rates and Hg bank concentrations related to longitudinal slope. Calibration was performed for both inorganic Hg and methyl-Hg water column concentrations using data collected during medium flows on June 16, 1994. Contribution of MeHg from bank erosion was calibrated with data collected during a higher flow event on June 10, 1995. Subsequent verification was accomplished using data from low (July 16, 1994 and July 23, 1997) and high (June 10, 1995) flow regimes. The model simulates inorganic mercury with an average root mean error of 153.8 ng/L (25% RME). Methylmercury concentration are simulated with an average RME of 1.08 ng/L (40% RME).

#### THE BERKELEY ARSENIC RESEARCH PROGRAM

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In 1989, a risk assessment project identified potential high cancer risks from arsenic in drinking water. A multidisciplinary research program was initiated to focus on identifying the cancers caused by arsenic ingestion and their dose-response relationships, investigations of arsenic methylation, and studies of cellular and molecular biological markers of effect. The core funding for the research program came from the Superfund Program, but additional research project funding has been received from the NIEHS and the U.S. EPA. Current projects include studies in California, Nevada, Argentina, Chile, and India. Major collaborating centers include the University of Washington, Seattle, UCSF, the Universidad Catolica in Cordoba, Argentina, GREDIS in Santiago, plus collaborators in Antofagasta in Chile, and the Post Graduate Medical Institute in Calcutta, India. Our projects have shown that ingestion of arsenic in drinking water is a cause of bladder and lung cancer, and probably also kidney cancer. The metabolic studies have shown that inorganic arsenic is incompletely metabolized at all levels of intake, and therefore protection by methylation from low doses of inorganic arsenic would not occur. The bladder cell micronucleus studies have shown effects at quite low intake of inorganic arsenic. Current studies in California, Nevada and Argentina focus on identifying doseresponse relationships for bladder cancer. We are also assessing genetic susceptibility and tumor DNA analyses aiming to identify mechanisms of arsenic carcinogenicity. We have commenced a study of arsenic-caused skin lesions in India examining the impact nutrition, including blood micronutrients. We are also planning a further mortality study in an area of Chile where we have shown that arsenic is causing more cancer deaths than cigarette smoking.

#### PORPHYRIN PROFILE AND CHELATOR CHALLENGE IN HG-EXPOSED VOLES

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Porphyrins occur in characteristic profiles in the liver, kidney and urine. Changes in profiles of the six measurable porphyrin intermediates, particularly the 4carboxylporphyrin (Copro) form, are indicative of mercury exposure and effect. Mercury chelation with 2.3-dimercaptopropane-1-sulfonate (DMPS) and subsequent measurement of urinary Hg and porphyrin profiles more accurately approximates tissue burdens of Hg and their effects. We determined an appropriate DMPS dose in the prairie vole (Microtus ochrogaster). Voles received 1 ppm methyl mercury in drinking water for four weeks after which they were challenged with saline, 0.01, 0.1 or 1.0 mmol/kg DMPS, ip. Urine was collected before and after saline or DMPS injection. Mercury reached 1.4 ppm in the brain (92% organic) and 15.5 ppm (94% organic) in the kidney. DMPS reduced brain Hg levels to 0.98, 1.3 and 1.1 ppm and kidney Hg levels to 7.1, 7.6 and 9.5 ppm in the high, medium and low DMPS treatments, respectively. Urinary Hg levels (24 hr. total) increased from 1.63 to 11.7, 8.04 and 3.47 µg in the high, medium and low DMPS treatments, respectively (~65% organic Hg). Methyl mercury increased renal levels and urinary excretion of Copro. DMPS decreased renal and urinary Copro. These data support the use of the combined porphyrin profile / DMPS chelation technique, establish an appropriate DMPS dose, and demonstrate that brain mercury concentrations were not increased by DMPS administration in voles. Funded by NIH-ES04696.

# EFFECT OF TCDD ON IN VITRO STEROIDOGENESIS BY CHICKEN OVARIAN FOLLICLES

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A growing body of research supports the effects of halogenated aromatic hydrocarbons on endocrine systems in both mammals and birds, including multigenerational effects on reproduction and behavior. However, their effects on ovarian steroidogenesis in birds have not been previously studied. Therefore, this experiment utilizes an *in vitro* model of chicken ovarian follicles in culture to address this question utilizing TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) as the model compound.

Ovarian follicles from laying Rhode Island Red hens are dissected and granulosa and thecal cells cultured separately on 24 well plates with 1 mL of Medium 199 supplemented with 5% calf serum and 1% antibiotic-antimycotic. Media is collected before and after treatment and frozen until assayed for estrone and progesterone production using an enzymeimmunoassay. Cells are treated with media alone, TCDD, or equal amount of vehicle with four replicates each.

Preliminary results show that TCDD exposure at 10<sup>-7</sup>M significantly decreased progesterone production within 4 hours. Further experiments will clarify the dose-response and time-course relationships between dioxin exposure and progesterone levels in addition to determining the specific event(s) impacted.

# REPRODUCIBILITY OF THE MTHFR LOCUS AS A MARKER FOR LEUKEMIA RISK IN CHILDREN

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INTRODUCTION: A genetic polymorphism in the enzyme 5, 10-methylenetetrahydrofolate reductase (MTHFR) leads to decreased levels of activity in this enzyme, which may be associated with increased DNA damage. Given the importance of chromosomal changes in childhood leukemias, we postulate that the MTHFR gene polymorphism may be an important susceptibility marker for childhood leukemia. We have conducted some preliminary laboratory analysis to evaluate the use of this marker.

METHODS: Two phases of a three phase process were conducted to determine whether genotyping results can be replicated in two labs. The genotype was classified as 0=wildtype, 1=heterozygote, 2=variant, and 3=inconclusive. Phase I: Ninety-eight samples of peripheral blood (laboratory 1) or buccal cells (laboratory 2) were selected for genotyping in separate labs to determine repeatability of results. Biologic material from 60 subjects was analyzed by both labs. Phase II: Due to insufficient material from buccal cell and peripheral blood specimen, 13 peripheral blood samples from laboratory 1 were provided to laboratory 2 and 11 buccal cell samples from laboratory 2 were provided to laboratory 1. Phase IIa: PCR images from the 17 common samples were reviewed by three readers in both labs to evaluate and standardize reader interpretation as an explanation for non-matches.

RESULTS: Phase I: Genotyping results for 41 out of 60 subjects matched (68%). Nineteen did not match (32%). After repeat analysis and removal of three recording errors, six out of 16 (37.5%) remaineded discrepant. Phase II: In laboratory 2, two out of 17(13 subjects, plus four seeded samples) were non-matches. Laboratory 1 could not analyze 11 samples from Laboratory 2. Phase IIa: There was complete inter- and intra- laboratory agreement interpreting the PCR images.

DISCUSSION: Out of 29 samples re-analyzed (16 non-matches and 13 from laboratory 2), eight remain discrepant (28%). Sources of variation in results, such as incomplete digestion by the restriction enzymes, will be explored in Phase III of this reproducibility study. Genotypes coded as inconclusive will be traded to the other lab for analysis. Reproducibility will be tested on another gene (NQ01) to see if similar results emerge.

CONCLUSION: In molecular epidemiology, it is important to identify, describe, and alleviate sources of inter- and intra- laboratory variability when using different laboratories and different sample media, especially if results are to be used as the basis for epidemiologic inference.

# MTBE WEBSITE HOSTED BY UNIVERSITY OF CALIFORNIA TOXIC SUBSTANCES RESEARCH AND TEACHING PROGRAM

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Of the six MTBE research proposals funded by the UC Systemwide Toxic Substances Research & Teaching Program (TSR&TP) under Senate Bill 521, the proposal from UC Davis included co-funding from the National Institutes of Environmental Health Sciences (NIEHS) Superfund Basic Research Program at UC Davis for a public outreach segment.

One of the goals of the public outreach effort was to establish an MTBE website to help disseminate disinterested third-party information about MTBE and to provide updates on MTBE-research at UCD to the public. The outreach effort also has as its goal, provision of information for junior and senior high school students for projects. An experiment on MTBE taste and odor in drinking water that is suitable for a high school science class is currently being developed and will be available to interested teachers by November, 1998. The experiment will illustrate criteria used in generating drinking water standards, as well as principles of toxicology and comparative risk.

The MTBE website is being hosted by the TSR&TP so that the public will be able to follow the UCD MTBE research and emerging MTBE issues.

Visit the UCD MTBE website at http://tsrtp.ucdavis.edu/mtbe.

#### GENOTOXIC INTERACTIONS OF COMPLEX PAH MIXTURES.

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Oily and wood-preserving chemical wastes have been identified in several thousand chemical waste dumpsites throughout North America. These chemical wastes contain complex mixtures of several different classes of halogenated aromatic hydrocarbons (HAHs) and polynuclear aromatic hydrocarbons (PAHs) which include: polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), diphenyl ethers (PCDEs), biphenyls (PCBs), benzenes (CBs), and phenols (CPs), hydroxy PCDEs, and complex PAH mixtures containing diverse 2-, 3-, and > 4-ring congeners. Hazard and risk assessment of HAHs and PAHs associated with individual sites before and after remediation is difficult due to the complex mixtures of toxic chemicals which have been identified at these sites. This program has developed a battery of class-specific bioassays for HAHs and PAHs which can be used directly for quantitative risk assessment of complex mixtures derived from various sites. Tests have been developed to utilize a variety of cells in culture to measure the gentoxicity, developmental toxicity and immunotoxicity of various samples. Using this battery of bioassays, individual compounds, reconstituted mixtures, and isolated fractions can be evaluated using the different toxicological endpoints. Two PAH mixtures extracted from Manufactured Gas Plant Residue (MGPR) were fractionated using Thin Layer Chromatography (TLC). This extraction method generated four fractions with fraction 1 containing primarily naphthalene and methylnaphthalene, fraction 2 containing fluorene, alkylfluorene isomers, anthracene and phenanthrene, fraction 3 containing alkylphenanthrenes, fluoranthrene, benz(a)anthracene, benzo(a)pyrene (BAP) and perylene, and fraction 4 containing compounds with more than 4 fused-aromatic rings. In a previous study, a 250 µg dose of BAP produced a tumor incidence of approximately 40% in male B6C3F1 mice, whereas an extract of MGPR-4 induced approximately 45% tumor incidence, although the mixture contained only 14 ppm BAP. When MGPR-4 was separated into four fractions using TLC, fractions 3 and 4 induced a genotoxic response of over 1,300 revertants per mg; however, the BAP content of fraction 3 was 26.6 ppm, while fraction 4 was found to contain 0.88 ppm BAP. These results suggest that components of fraction 4 other than BAP were capable of inducing a genotoxic response. Another sample of MGPR was also separated using TLC. Fraction 2 extracted from MGPR-1 induced 300 revertants at a dose of 1 mg per plate, while fraction 3 induced 1,399 revertants per mg. Studies are underway to utilize HPLC fractionation to isolate the low molecular weight PAHs, alkylPAHs and high molecular weight PAHs for analysis. The data from these studies should be of value for improving the accuracy of Toxic Equivalency Factors in risk assessment. This program is supported by the National Institute of Environmental Health Sciences Grant No. P42 ES04917-08.

### AMMONIA DETECTION AND MONITORING USING PHOTOFRAGMENTATION-FLUORESCENCE

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Ammonia injection is widely used to reduce nitrogen oxides from industrial boilers and furnaces in both the selective catalytic reduction (SCR) and selective non-catalytic reduction (SNCR) processes. In the SNCR process, NH<sub>3</sub> is injected in a carefully-controlled temperature window (typically around 1300 K, or lower with the concurrent injection of H<sub>2</sub> or some other radical source) that promotes the breakdown of NH<sub>3</sub> to NH<sub>2</sub>, followed by reaction of NH<sub>2</sub> with NO to form N<sub>2</sub>H, and finally, molecular nitrogen. The kinetics of the SNCR process are extremely temperature-sensitive; if the temperature is too high, NH<sub>2</sub> is oxidized to NO, while at lower temperatures the radical pool is too small for the initiation step (typically NH<sub>3</sub> + OH  $\rightarrow$  NH<sub>2</sub> + H<sub>2</sub>O) to proceed. In either case, outside of the correct temperature window, the result is the net generation of NO, either in the hot exhaust or in the atmosphere following emission of NH<sub>3</sub>.

Excimer laser fragmentation-fluorescence spectroscopy (ELFFS) is shown to be an effective detection strategy for NH<sub>3</sub> in combustion exhausts at atmospheric pressure and high temperatures. Two-photon photofragmentation of NH<sub>3</sub> with 193 nm light yields emission from the NH (A-X) band at 336 nm. There are no major interferences in this spectral region, and the sensitivity is at the ppm level. In typical combustion monitoring situations, the NH fluorescence signal is not significantly affected by changes in temperature or equivalence ratio. Quenching of the NH(A) state radical by the major combustion products is measured, and does not limit the applicability of the detection method. Single-shot detection limits in realistic environments are below 1ppm; the 100-shot (1 second) detection limits for NH<sub>3</sub> in 450 K post-flame gases are below 100 ppb. This technique could prove useful in monitoring ammonia emissions from catalytic and non-catalytic NO<sub>x</sub> reduction processes.

#### LDI TOF-MS ANALYSIS OF CHROMIUM CONTAINING PARTICULATES.

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Chromium is widely used in alloys, electroplating, leather tanning and dyes. Emissions of chromium into the atmosphere result from these activities and from incineration of wastes containing chromium. The toxicity of chromium depends on the oxidation state; where the hexavalent form of chromium (Cr[VI]) is known to be highly carcinogenic, while the trivalent form of chromium (Cr[III]) is non-carcinogenic and an essential nutrient for humans. Combustion of chromium compounds results in the formation of an aerosol containing micron and submicron particles which can stay airborne for long periods of time and penetrate deeply within the lungs, and thus have the potential for mutagenic and carcinogenic effects.

Laser desorption ionization (LDI) time-of-flight mass spectrometry (TOF-MS) has been used for examining the fate of chromium in a model incinerator. The coupling of lasers as a ionization source with mass spectrometric techniques provides molecular, fragment and cluster information about inorganic materials which is not easily obtained by conventional techniques. LDI TOF-MS offers the advantages of minimal sample handling and preparation which leads to cost-effective analysis times.

Chromium aerosols are difficult to analyze because some forms (e.g. Cr2O3) are insoluble and resistant to digestion, even when subjected to vigourous conditions such as HNO3/H2O2 digestions. As a result, chromium recoveries are often poor when Cr is analyzed using atomic absorption spectroscopy or ICP-AES.

#### BIOMARKERS OF GENETIC DAMAGE IN CHILDREN OF THE INNER CITY

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Children may be more susceptible to the effects of environmental pollution than adults. While all children are sensitive to environmental hazards, minority children and children of lower socioeconomic status are particularly at risk because they tend to live in less healthful environments, which may include both pollution and inadequate diet.

However, there are few studies which examine biomarkers of genetic damage in children, especially in epithelial cells. We are attempting to find out whether children and adults of the inner city have a higher risk of folate deficiency due to poor diet, and an increased prevalence of genetic markers which have been associated with cancer. The objective of this study is to collect data on the background levels and variability of cytogenetic and molecular biomarkers in the exfoliated cells and blood of children.

Buccal, urothelial, and blood cells were collected from African-American children 4-12 years of age, and from their parents or an adult living in the same household. This study design allows comparison of biomarkers in adults and children of the same ethnic origin residing in the inner city of East Oakland. Among fifty families, approximately half live less than three blocks from major freeways or gas stations. The spectrum of molecular and cytogenetic biomarkers to be examined includes micronuclei, folate and B<sub>12</sub> levels, DNA polymorphisms, cotinine, and t,t-muconic acid measurements. Preliminary analysis with a limited number of samples shows similar levels of cytogenetic damage in children and adults of the inner city. New data will be presented, and results of the study will allow for the assessment of the utility of using these biomarkers to examine genetic damage in children.

### A COMPARISON OF CYTOGENETIC DAMAGE IN HUMAN HEMATOPOIETIC CD34+ PROGENITOR CELLS AND PERIPHERAL BLOOD LYMPHOCYTES.

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Micronuclei (MN) have been used as a biomarker of cytogenetic damage and are easily measured under fluorescent microscopy. They consist of small amounts of DNA that arise in the cytoplasm when chromatid/chromosomal fragments or whole chromosomes are not incorporated into daughter nuclei during mitosis. We have used the MN assay to assess the baseline level of cytogenetic damage in progenitor cells from human bone marrow (BM) and umbilical cord blood (CB) enriched for CD34 antigen. It has been postulated that hematopoietic progenitor cells may be less susceptible to cytogenetic damage than other circulating cells. If these cells are at lower risk for genetic damage, a decreased incidence of MN would be expected. To our knowledge there have been no previous studies using the MN assay on human BM or CB cells, nor in a progenitor Using a two-step immunomagnetic bead separation system enriched population. (Miltenyi Biotech, Inc, CA), BM and CB progenitor cells were enriched for CD34 antigen. The purity of CD34+ in the enriched population was verified by FACScan analysis. The average CD34 antigen purity achieved for our BM samples was 62% (range 22-96%) and for CB was 71% (range 61-80%). The isolated BM and CB progenitor cells were cultured for 72-96 hours in a media enriched with an optimized combination of cytokines. Cytochalasin B was added 44-68 hours post-initiation as described by Fenech and Morley (Mutation Res. 147, 29-36, 1985). Cytochalasin B prevents the cells from completing cytokinesis resulting in the formation of binucleated cells. Cells were analyzed for the MN frequency under fluorescent microscopy as described by Yager et al. (Cancer Res. 50, 393-399, 1990). Preliminary data on eight BM samples reveal a similar MN frequency to that in previously published data on peripheral lymphocytes. A lower MN frequency was noted in five CB samples when compared to BM progenitor cells and peripheral lymphocytes, which is consistent with previous data showing that MN frequency is age-related. We report the first use of micronuclei as biomarkers for cytogenetic damage in BM and CB CD34 antigen enriched populations. This is an important advancement in the application of the MN assay because the BM and CB hematopoietic progenitor cells are the likely target cells in the development of leukemias and also in the development of various BM disorders. Further studies with chemical exposures are underway to evaluate differential sensitivity to in vitro cytogenetic damage.

### Selected Restaurants in the Berkeley Area

Bay Wolf Chef Lauren Lyle features a menu from a different region of the Mediterranean

every three weeks at this comfortable restaurant. - Michael Bauer

credit cards accepted · beer-wine only

(510) 655-6004 3853 Piedmont Ave. Oakland

Lunch weekdays. Dinner nightly. Reservations: Recommended

Cafe at Chez Panisse

Located above the landmark restaurant, this casual cafe always features great pizza and the absolute best garden salad on the daily changing menu. - Michael Bauer credit cards accepted beer-wine only

(510) 548-5049 1517 Shattuck Ave., Berkeley Lunch and dinner Mon.-Sat. Reservations: Accepted

Cesar

Excellent tapas are well priced in this rustic, homey dining room. Sophisticated bar, great wine list.- Michael Bauer credit cards accepted full bar open late outdoor seating

(510) 883-0222 1517 Shattuck Ave

Berkeley

Parking: Fairly easy street parking

3 p.m.-midnight daily Reservations: No

Ginger Island

East-West menu is back on track but tropical menu feels dated. Good chicken brochettes and ginger drinks.- Robin Davis credit cards accepted · full bar

(510) 644-0444 1820 Fourth St. Berkeley ·

Lunch and dinner daily Reservations recommended

Jordan's (at the Claremont Hotel)

Service and the dining room's bay view outshine the dishes, which can be muddled with too many flavors and poor execution.- Robin Davis credit cards accepted · full bar

(510) 843-3000

41 Tunnel Road (at Ashby), Berkeley

Mon.-Sat.; din. daily; brunch. Sun.

Reservations

### Garibaldis on College

Comfortable, sophisticated atmosphere and good service. The Mediterranean dishes with East-West Oakland accents are often excellent even though a little Parking: uneven. - Michael Bauer

(510) 595-4000 5356 College Ave., Oakland Lunch weekdays, Dinner nightly Reservations: Accepted

Kirala

Delicious grilled morsels from the robata grill at reasonable prices.

Fantastic sushi. No reservations can mean torturous waits- L H. beer-wine

only

(510) 549-3486

2100 Ward St., Berkeley

Lunch Tues.-Fri., dinner nightly

Reservations: No

Oliveto

Paul Bertoli serves the best Italian food in the Bay Area. Benchmark fresh pastas, grilled pigeon, veal Milanese. Casually professional service.-

Michael Bauer credit cards accepted · full bar

(510) 547-5356

5655 College Ave., Oakland

Lunch Mon.-Fri., dinner nightly Reservations: Recommended

Pyramid Brewery & Alehouse

Go for the good beers on tap, the fun ambiance and crowd, the cheerful, enthusiastic staff. Don't expect much from the food.- Maria Cianci credit cards

510

accepted · beer-wine only

(510) 528-9880

901 Gilman Street (near 9th St.)

Berkeley

Parking: Parking lot is attached to the complex

Lunch, dinner daily Reservations: Accepted

Yoshi's

Stylish Japanese dining room and jazz club under one roof. Good sushi and

appetizers, weak desserts.- Robin Davis credit cards accepted full bar

(510) 238-9200

Embarcadero West (near Washing St. in Jack London Square), Oakland

Lunch, dinner ton daily

Reservations: recommended

Zachary's Chicago Pizza

For stuffed pizza enthusiasts, this is the place. Thin crust pizza excellent, too. But a mob-like atmosphere makes it

better to get the pies to go.- L.H.

(510) 655-6385 5801 College Ave., Oakland

Reservations: No

Superfund Basic Research Program - Annual NIEHS/EPA Superfund Research Meeting EPA/Tri-University Superfund Research Programs: Partnership for a Clean and Healthy Environment	October 26-27, 1998
Conference Notes	
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